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STUDIES ON TRANSMISSION OF THE TRISTEZA VIRUS BY THE VECTOR, APHIS CITRICIDUS

A. S. Costa and T. J. Grant[1]

(Accepted for publication September 14, 1950)

The first evidence that the tristeza disease of citrus is caused by a virus was obtained by Meneghini (6), who reported its transmission by the aphid, *Aphis tavaresi* Del Guercio. Bennett and Costa (1, 2) confirmed Meneghini's results on transmission of tristeza by the oriental citrus aphid, but these authors referred to the insect under the name *Aphis citricidus* Kirk., since it seems that *A. tavaresi* and *A. citricola* Van der Goot are synonyms for that species.

In Argentina, where tristeza or "podredumbre de las raicillas" appeared before it was present in Brazil, the vector has been reported under the name *Paratoxoptera argentinensis* Blanchard (10). There is no doubt that the insect vector of the tristeza disease in Argentina and that in Brazil are the same,[2] but further taxonomic studies seem necessary before a decision can be reached concerning the best name for this species complex, which includes *Aphis citricidus, A. tavaresi, A. citricola,* and *Paratoxoptera argentinensis.* Under one of these names, this insect has been reported occurring in most citrus-growing regions of the world, including several countries in South America, Europe, Africa, Asia, and Indonesia.

The nearest place to the United States where this insect has been recorded seems to be Bermuda (8); *Aphis tavaresi* was reported there on *Bidens pilosa* L. This might be a questionable record of this aphid, since it has never been observed to occur on the same plant species in Brazil, where the insect and the plant are very common.

It is surprising that an insect so widespread in the world has not yet reached the citrus areas of the United States. Since a disease similar to tristeza, called quick decline, occurs in California and apparently spreads in a comparable manner, it might be postulated that the vector of the virus in that region is another insect. Recent results obtained by Dickson[3] indicate that *Aphis gossypii* Glov. can transmit the quick-decline virus, although this species seems to be a poor vector.

Extensive rootstock tests are being made cooperatively by the Instituto

[1] Respectively, Pathologist, Instituto Agronômico de Campinas, State of São Paulo, Brazil; and pathologist, U. S. Department of Agriculture, Bureau of Plant Industry, Soils, and Agricultural Engineering, Division of Fruit and Vegetable Crops and Diseases; Attaché, U. S. Embassy, Rio de Janeiro, Brazil.

The writers are indebted to Drs. R. C. Dickson, C. W. Bennett, B. A. Porter, and F. E. Gardner for their helpful comments during preparation of this manuscript.

[2] Specimens of *Aphis citricidus* from Brazil were sent to Dr. M. V. Fernandez Valiela, Ministerio de Agricultura de La Nacion, in Argentina, where the insects were compared with *Paratoxoptera argentinensis* and considered identical by Dr. Esmenia A. Tapia, Ministerio de Agricultura de La Nacion.

[3] Written communication made to the writers by Dr. R. C. Dickson, Division of Entomology, University of California, Citrus Experiment Station, Riverside.

Agronômico de Campinas and the U. S. Department of Agriculture, at Campinas, Brazil, to study the reaction of different citrus stock-scion combinations to tristeza (4, 5). It has been necessary to inoculate large numbers of plants by means of the vector, and the studies reported in this paper were made to gain more knowledge on the factors involved in the aphid transmission of the disease.

A good source of aphids was essential for use in the extensive field inoculations. After many trials it was determined that consistently good transmission could be secured with aphids reared on apparently healthy but virus-carrying Barão sweet-orange trees (*Citrus sinensis* (L.) Osbeck), budded on virus-tolerant Caipira sweet-orange rootstock. An orchard with about 150 trees of this combination was available at the Instituto Agronômico. Groups of 25 to 50 aphids collected from these trees transmitted the tristeza disease to approximately 75 per cent of the test plants. The trees of this orchard were used as a standard source of viruliferous aphids for most tests, except where otherwise indicated.

INSECT STAGES AND NUMBER OF INSECTS PER PLANT

After a suitable source of viruliferous aphids had been found, tests were made to determine the relative effectiveness of varying numbers of aphids in the transmission of the disease and also to determine whether nymphs, apterous adults, and alate adults differed greatly in their ability as vectors. The results are in table 1.

TABLE 1.—*Transmission of tristeza in relation to insect stages and to number of insects per test plant*[a]

Insect stage	Insects per plant	Plants		Transmission
		Inoculated	Infected	
	Number	*Number*	*Number*	*Per cent*
Nymph	25	14	7	50
Adult apterous	1	30	5	17
	5	7	2	29
	25	16	12	75
Adult alate	1	25	4	16
	5	10	1	10
	25	14	3	21
Mixed	50 to 100	30	23	77
	100 or more	60	53	88

[a] Test plants composed of a sweet-orange scion grafted onto sour-orange (*Citrus aurantium* L.) rootstock, prepared as described by Bennett and Costa (1).

The tristeza disease can be transmitted by single adult aphids of the winged and nonwinged forms. Inoculations with equal numbers of young nymphs and apterous adult aphids indicated the latter to be somewhat more efficient vectors. It also is evident that the percentage of transmission increased when larger numbers of aphids were employed. A minimum of

25 aphids per plant was necessary to obtain a high percentage of transmission.

In these as in other greenhouse and field tests the best results were secured with vigorous colonies of apterous adult aphids. Aphid-infested twigs were placed on the plants to be inoculated, and when leaves and succulent stems of the twigs wilted, the aphids crawled off and established themselves on the test plants.

FEEDING PERIOD REQUIRED BY VIRULIFEROUS APHIDS TO TRANSMIT THE VIRUS

Large apterous adults were transferred onto test plants by means of a moistened brush in all tests with feeding periods of 1 hr. or less. In tests of longer feeding periods the aphids were encouraged to crawl from the cut twigs onto the plant to be infested. The required number was usually obtained within 30 min. When the feeding period terminated, the aphids were brushed off the test plants by means of a dry cotton swab and the plants were then sprayed with a strong nicotine solution. The procedures employed were uniform and the feeding times serve as a basis for relative comparisons.

Data obtained in two tests show that groups of 25 to 50 viruliferous aphids were capable of transmitting tristeza when fed on test plants for 30 min. The percentage of transmission increased with longer feeding time, 4 hr. being enough to secure maximum transmission. In routine experiments, however, it was more convenient to leave the insects on the test plants for 24 hr.

RETENTION OF VIRUS IN THE VECTOR

The need of having an adequate supply of nonviruliferous aphids led to an attempt to determine whether viruliferous aphids could be rendered noninfective through feeding on healthy plant material.

Aphids collected from virus-carrying Barão sweet-orange trees were allowed to feed on healthy plants of sour orange, Sunshine tangelo (*Citrus paradisi* Macf. × *C. reticulata* Blanco), and "mandioquinha" (*Arracacha esculenta* DC.) for periods from 24 to 120 hr. A sample of the viruliferous aphids was used to inoculate test plants to serve as controls for the infectivity of the aphid collections.

Viruliferous *Aphis citricidus* did not remain infective after feeding on healthy plants for 24 hr. or longer. Also, the viruliferous aphids fed on the susceptible Sunshine tangelo for 5 days remained nonviruliferous. Since later tests showed that this plant had been infected, the result indicates that it takes more than 5 days for the virus to become systemic. This observation has been confirmed in other tests.

Detailed experiments have not yet been made to determine whether the aphid vector of tristeza can retain the virus when fed for periods shorter than 24 hr. on a series of susceptible test plants.

SOURCES OF NONVIRULIFEROUS APHIDS

Aphis citricidus breeds readily in cages if an adequate supply of fast-growing citrus plants is always at hand for successive transfers. It has been noticed, however, that unless the food plant is growing rapidly the colonies reared in cages are not so vigorous as those collected from field plants. Attempts made to rear this insect on plants outside the family *Rutaceae* have in general failed, although some breeding has occurred on a large-leaved species of *Oxalis* and on very tender shoots of the avocado plant (*Persea americana* Mill.).

Field colonies of aphids collected from sweet-orange plants in areas where tristeza is present have been tested and usually have been viruliferous. On the other hand, field collections of aphids obtained under similar conditions but from sour-orange plants or from Eureka and Lisbon lemon trees (*Citrus limon* (L.) Burm. f.), have been noninfective in the cases tested to date. Thus, in most tests in which great numbers of nonviruliferous aphids have been needed, field collections from lemon or sour-orange trees have been used after aphids were allowed to feed for 48 hr. on healthy citrus plants.

FEEDING PERIOD REQUIRED BY NONVIRULIFEROUS APHIDS TO BECOME INFECTIVE

A limited number of tests have been carried out to determine the feeding period required by nonviruliferous aphids to become infective; however, the tests have not been made for short feeding periods or with individual aphids, as would have been desirable. Also, no starvation of the aphids

TABLE 2.—*Feeding period required by nonviruliferous aphids to become infective*

Feeding time (hours)	Test plants infected out of 5 infested by groups of 25 to 50 nonviruliferous aphids fed on diseased twigs		
	Test 1	Test 2	Test 3
0.5	0	0	0
1	0	0	1
4	.....	2	1
6	.....	1	1
24	2	5	5

before or after the infection feeding has been attempted. In these tests the nonviruliferous aphids employed were reared in cages or were field collections of aphids that had been fed on healthy citrus plants for 48 hr.

The nonviruliferous aphids were placed on cut succulent twigs of Barão sweet orange known to be carrying the tristeza virus. They were allowed to feed for varying periods and were subsequently transferred to healthy test plants by means of a fine brush and allowed to feed for 48 hr. The results are in table 2.

Nonviruliferous aphids can occasionally become infective in 1-, 4-, or

6-hr. feeding periods as tested with groups of 25 to 50 insects. Their ability to become infective is materially increased with a longer feeding period.

RECOVERY OF TRISTEZA VIRUS FROM INOCULATED PLANTS, BY APHIS CITRICIDUS

Test plants of sweet-orange over sour-orange rootstock require from 1 to 3 months from date of inoculation with viruliferous aphids to show yellowing of young leaves, stunting, and the other symptoms of tristeza (Fig. 1).

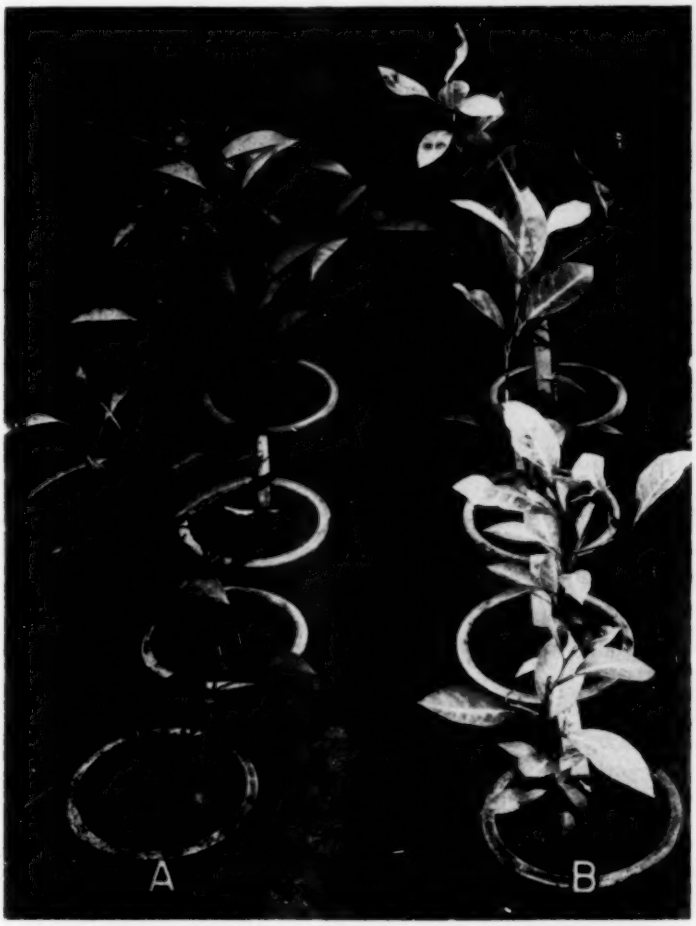

FIG. 1. Budded sweet-orange plants grafted on sour-orange rootstock. A. Healthy plants. B. Plants inoculated by means of *Aphis citricidus*, about 4 months after inoculation.

On the basis of external symptoms it is obvious that the virus must have multiplied and become systemic in the plant prior to the onset of the symptoms. It seemed desirable, therefore, to determine how long it is after a plant is inoculated until the virus can be recovered by means of the vector.

Viruliferous aphids fed on susceptible healthy plants lost the virus and

did not become infective again even after 5 days of continuous feeding on the same plant. On the basis of this experiment it was decided to make the tests for recovery of the virus at 5-day intervals.

Groups of 100 viruliferous aphids were used to inoculate 25 young test plants, 8 to 10 in. high, of sweet-orange over sour-orange rootstock. On five plants the aphids were allowed to feed for 5 days and were then transferred to a new series of five healthy test plants. On the other 20 inoculated test plants the original viruliferous aphids were allowed to feed for 48 hr. and were then killed with a nicotine spray. On the 9th, 14th, 19th, and 24th days following inoculation, about 100 nonviruliferous aphids were placed on each of five of the remaining inoculated plants, allowed to feed for 24 hr., and then transferred to five healthy test plants. After feeding on these for 48 hr. they were killed.

No virus could be recovered from the inoculated plants 5 days after inoculation; 10 days after inoculation the virus was recovered from two of five inoculated plants, and thereafter the virus could be recovered from practically all of the inoculated plants. These results indicate that systemic invasion of the virus might occur between 5 and 10 days after inoculation by the vector; and that 10 days after inoculation the virus attained a degree of concentration in the young leaves of the sweet-orange scion that permitted the aphids feeding on them to become viruliferous.

RESISTANCE TO INFECTION BY APHIDS

As previously reported (2), under field conditions the sour-orange plant, on its own roots or budded on another sour-orange plant, is resistant to tristeza. Also, in field tests in which a total of 66 sour-orange plants were inoculated three times, only 10 showed symptoms (3). In greenhouse tests, however, in which sour-orange seedlings were inoculated comparatively by aphids and by approach grafts, the second method of inoculation proved more successful. In other tests plants of sour orange with a mixed top, composed of a sweet-orange branch and a sour-orange branch, were more easily infected by the vector than were comparable plants of sour orange without the sweet-orange branch. This resistance of the sour orange when inoculated by the vector might be similar to that reported for certain potato varieties (9) which have a field immunity for virus A but can be infected by grafting.

The tristeza virus has been recovered from infected sour-orange plants by grafts, but not by means of the vector. Low virus concentration in the plant or inability to feed in the tissues where virus is present might explain why the vector does not pick up the virus from the sour-orange plant.

TRANSMISSION BY OTHER INSECTS

In addition to the results of Bennett and Costa (2), tests were made with six species of aphids,[4] *Aphis fabae* Scolopi, *A. gossypii* Glov., *A. medi-*

[4] The authors are indebted to Dr. R. C. Dickson, University of California Citrus

caginis Koch, *Macrosiphum ambrosiae* Thos., *M. solanifolii* (Ashm.), and *Toxoptera aurantii* Koch; one coccid, *Coccus viridis* (Green); and a psyllid, *Diaphorina citri* Kuwayama.

The insects were fed on virus-carrying Barão sweet-orange twigs for 24 hr. or longer and then transferred onto test plants of sweet-orange on sour-orange rootstock. A minimum of 100 insects per plant was used in case of aphids, and from 25 to 50 insects for the other species.

None of the insects tried transmitted the tristeza virus, although the number of tests made with some species was small and did not permit definite conclusions.

DISCUSSION

In the early reports of transmission of tristeza by aphids, Meneghini (6) and Bennett and Costa (1) employed large numbers of insects per plant in order to insure transmission. Bennett and Costa (2) reported later that the use of 50 aphids per plant was less successful than 300, but that transmission had been accomplished by individual aphids in two out of 30 plants inoculated.

The results of the present investigation confirm the fact that large numbers of aphids are more effective than small numbers in transmitting the disease. They also indicate that groups of 25 to 50 aphids fed on a good source of inoculum, such as virus-carrying Barão sweet-orange trees budded on a tolerant rootstock, transmit the disease in approximately 75 per cent of the cases. The data also show that the disease was transmitted by single aphids, both winged and nonwinged forms. The fact that single, winged aphids are capable of transmitting tristeza explains how the disease could spread throughout the State of São Paulo in less than 10 years and destroy upward of 6,000,000 trees of commercial citrus varieties budded on sour-orange rootstock. The knowledge that single aphids are able to transmit tristeza, considered with the evidence presented by Meneghini (7) that aphids starved for 24 and 48 hr. might still be infective, is of utmost importance in relation to quarantine measures and air-travel inspection. It shows how it might be possible for the disease to gain entrance in most citrus-growing areas of the world, since a single viruliferous aphid could be carried in an airplane from an infested area to almost any region in the world within 24 hours.

In transmission studies of tristeza by means of its vector it is important to have virus-carrier trees, such as sweet orange budded over a tolerant rootstock, on which viruliferous aphids can be reared. It is not yet known whether carrier trees have a higher virus concentration than plants budded on nontolerant rootstocks, but since the aphids breed and feed well only on new growth, plants with tristeza symptoms are usually in a poor state of growth and are not favorable hosts for the vector.

Experiment Station, for identification of the aphids; and to the Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture, for identification of the species *Diaphorina citri*.

In tests to determine the feeding period required by viruliferous aphids to transmit tristeza, groups of 25 to 50 aphids could transmit the virus in 30 min., but the percentage of transmission was higher when the aphids were left on the plants for longer periods. It is not known whether the aphids that remained on the test plants for longer periods were more effective because of the increased feeding time of individual aphids, or because a larger number of aphids in the groups had more chance to feed.

The information now available does not permit a definite conclusion as to whether the tristeza virus is persistent or nonpersistent in the insect vector, *Aphis citricidus*. The results of Meneghini (7) on retention of the virus by fasting insects and the present results on retention of the virus by feeding aphids show that the virus is lost more rapidly by feeding than by fasting insects. They also show that the virus is not retained by feeding insects for 24 hr. These are characteristics of nonpersistent viruses (11). On the other hand, the lack of effect of starvation prior to infection feeding on infectivity of the vector (2), together with the results showing that transmission increased with an increase in feeding time on test plants, and that infectivity of the aphid vector increased with an increase in the feeding time on the source of virus, all would suggest that the virus might belong to the persistent group. Watson (12), however, has indicated that some of these results might also be obtained with nonstarved aphids in the transmission of nonpersistent viruses.

When feeding viruliferous aphids on healthy citrus plants to render them nonviruliferous, there is a time limit beyond which the aphids could again pick up the virus from the host plant. Viruliferous aphids, fed for 24 hr. up to 5 days on healthy Sunshine tangelo and on sour-orange seedlings, became noninfective. Recovery of the virus from the plants on which the aphids were fed was attempted 60 days later by means of groups of nonviruliferous aphids. The nonviruliferous aphids fed on Sunshine tangelo became infective, whereas those fed on the sour-orange seedlings did not. This is additional evidence that the sour-orange plant is resistant when inoculated by aphids. It also suggests the use of this plant for feeding viruliferous aphids for the purpose of obtaining nonviruliferous insects.

The tests of transmission of tristeza by insects indicate that *Aphis citricidus* is the natural vector of the disease in the State of São Paulo, although it is entirely possible that eventually some other insect vector may be found. Such vector may even be among those already tested, but if this is found to be the case its efficiency in transmission may be expected to be appreciably less than that of *Aphis citricidus*.

SUMMARY

A single adult of *Aphis citricidus*, either the apterous or the winged form, was capable of transmitting the tristeza virus. The percentage of transmission was greater when a good source of inoculum was established and when large numbers of aphids were employed.

Groups of 25 to 50 viruliferous aphids were capable of transmitting tristeza in periods of feeding as short as 30 min., and a feeding period of 4 hr. appears to be enough to insure maximum transmission.

Groups of 25 to 50 nonviruliferous aphids may become infective after feeding for 1 hr. on a diseased plant, but infectivity of the aphids increased with a longer feeding time on a diseased plant. The best transmission was with aphids that had fed for 24 hr. on the source of virus.

Viruliferous aphids become noninfective after feeding 24 hr. or longer on healthy plant material, and they remain noninfective if kept on the same plant for 5 days, even on citrus plants in which the virus is able to multiply. To obtain colonies of nonviruliferous aphids from field collections it is considered safer, however, to feed the aphids on healthy sour-orange plants.

The sour-orange plant was more easily infected with the tristeza virus when inoculated by grafting than by viruliferous aphids. The tristeza virus has been recovered from sour-orange plants by grafting, but not by means of the aphid vector.

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A MILD STRAIN OF THE TRISTEZA VIRUS OF CITRUS

THEODORE J. GRANT[1] AND A. S. COSTA[2]

(Accepted for publication September 14, 1950)

When it was demonstrated that the tristeza disease of citrus is caused by a virus, the finding of strains of the tristeza virus became theoretically possible. The relation of this disease in Brazil to similar disorders in other parts of the world has been the subject of considerable speculation (1, 6, 7, 8, 9). Bennett and Costa (1) noted, however, that despite striking similarities, certain variations in effects produced in different parts of the world suggest that the diseases are not identical in all instances. They suggested, on the basis of information then available, that if the disorders are not caused by the same virus it must be assumed that they are caused either by closely related viruses or by virus strains that vary in virulence and possibly in other characteristics. The latter alternative seemed to them the more tenable.

In the extensive rootstock tests being carried out cooperatively by the Instituto Agronômico de São Paulo and the United States Department of Agriculture at Campinas, Brazil, more than 2000 citrus rootstock-scion combinations have been inoculated with the tristeza virus. Numerous differences in various rootstock-scion responses have been noted (2, 3, 4), under reasonably comparable environmental conditions within the same field.

FIRST INDICATION OF STRAINS OF TRISTEZA VIRUS

In January 1948, when budding work was first started in the rootstock tests, it was decided that one source of buds should be the apparently healthy but virus-carrying Barão sweet-orange trees on the tolerant Caipira sweet-orange rootstocks in the orchard of the Instituto Agronômico. These are designated as Barão B buds. Five trees in this orchard were selected as a standard source of budwood and one bud of each tree was budded onto one plant of each of the citrus varieties in the rootstock test planting. Thus each rootstock had five plants with scion growth from virus-carrying Barão B buds. The designation Barão A buds is used for those taken from Barão sweet-orange tops of plants in the stock-scion test that had been grown from buds from healthy Barão seedlings and inoculated three times by means of viruliferous aphids.

As the virus-carrying Barão B buds developed, it was repeatedly observed that these buds over the tolerant rootstock varieties had no symptoms or other indication of the tristeza virus, but that over nontolerant rootstocks

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the growth from these Barão B buds invariably had symptoms of tristeza disease. Periodic notes were taken as to the severity of definite disease symptoms, graded numerically from 1 to 5, class 5 including severely diseased plants with leaf drop and dying of terminal growth; 0 indicated apparently healthy growth. By April 1948 it became apparent that growth from one of the Barão B buds over the nontolerant rootstock Watt tangelo was markedly different in that it had no disease symptoms, whereas the growth from the other four Barão B buds over the Watt tangelo rootstocks was noticeably diseased.

It did not seem possible that this exceptional plant had escaped infection inasmuch as its top growth was from a bud taken from a tree known to be carrying the virus. Also it had been inoculated at the end of January, as had all other plants in the rootstock test, by means of aphids obtained from a standard source of virus-infected trees. However, as a further test of its reaction to the tristeza virus, this exceptional plant was reinfested with viruliferous aphids in April, in August, and again in October. By January of 1949 this plant had only questionable or extremely mild tristeza symptoms; thus its reaction could definitely be considered as exceptional.

There appeared to be three possible explanations for the behavior of this plant: 1) In the rootstock test the plants being employed as rootstocks were of seedling origin and, inasmuch as the Watt tangelo is a hybrid, the seedling used as a rootstock for the exceptional plant might possess a high degree of tolerance to the tristeza virus; if so, it would have only very mild symptoms or none, even though infected with the tristeza virus. 2) Possibly the particular Barão B bud was a mutation and was characterized by a plant response that might prevent entry or development of the tristeza virus; thus the plant would remain apparently healthy. Or, 3) the exceptional plant had a mild strain of the tristeza virus that was protecting it against the severe strain, so that even though inoculated several times it would not show severe symptoms of tristeza disease.

ROOTSTOCK INFLUENCE

In order to determine whether the particular rootstock of the exceptional plant was or was not an important factor in the lack of tristeza disease symptoms, the following test was made. In January 1949, buds from the selected plant with questionable or mild symptoms were budded over five rootstock plants of the common Brazilian sour orange. At the same time, and for comparison, five other Brazilian sour-orange plants were budded with buds obtained from the trees in the Instituto orchard that were the standard source of virus-carrying Barão B buds. The initial growth of all buds was normal, as was to be expected from previous experience (2, 3, 4). At the end of 4 months, however, the differences between the growth from the buds from the selected plant and that from the virus-infected buds from the orchard were very marked. The latter had typical symptoms, whereas the bud growth from the selected plant had only ex-

tremely mild symptoms or none. These differences continued to become more distinct. In figure 1, A, photographed 7 months after budding, two

FIG. 1. Sweet-orange scions on Brazilian sour-orange rootstocks, infected with severe (s) and mild (m) strains of the tristeza virus. A, growth of infected buds. B, plants infected by aphid inoculation. C, plants infected with severe and mild strains, and (n) noninoculated control.

plants (5) have class 5 disease symptoms: all leaves have dropped and tip dieback has begun. The two plants at the right (m) are still apparently healthy; their scion growth is from buds of the plant selected as having extremely mild symptoms.

Since these very obvious differences in symptom expression occurred on plants having comparable nontolerant Brazilian sour-orange rootstocks, it is evident that the differences in symptoms were due not to any rootstock influence but to some other factor, possibly a special reaction of a bud mutation or the presence of a mild strain of the tristeza virus.

TRANSFER OF VIRUS FROM THE SELECTED PLANT WITH MILD SYMPTOMS

In order to eliminate, as far as possible, any influence of difference in symptom expression that might be due to the possible factor of bud mutation, and at the same time to test for the presence and transmissibility of a mild strain of the tristeza virus, the following tests were undertaken.

Nonviruliferous aphids were fed for 24 hr. on the original plant selection having extremely mild symptoms in the Barão sweet-orange top over the Watt-tangelo rootstock. Succulent, aphid-bearing twigs were then cut from the plant and placed on five healthy potted test plants composed of a sweet-orange (Florida sweet seedling) top and a Brazilian sour-orange

TABLE 1.—*Tests for transmission of virus from plants with mild tristeza disease symptoms, in comparison with transmission from a standard source of severe tristeza virus. Five plants in each treatment*

Test No.	Symptoms on plants used as virus source	Severity of symptoms[a] on 5 test plants				
		1	2	3	4	5
1	Mild	m	0	0	m	0
1	Severe	4	0	3	4	4
2	Mild	m	0	m	m	m
2	Severe	2	3	0	2	–

[a] 0, apparently healthy; m, very mild symptoms; 1 to 5, grades from definite initial to severe symptoms; –, tree died from unknown cause.

rootstock. The aphids, about 100 per test plant, were allowed to crawl off the cut twigs onto the test plants and feed for 24 hr. Viruliferous aphids from the standard source of virus-infected Barão sweet orange trees in the Instituto orchard were placed in the same way on another series of five comparable test plants. All aphids were killed with a nicotine spray after feeding for 24 hr. on the healthy test plants. The plants were then moved from insect cages to the screenhouse. This test was repeated 1 month later, the original plant selection and the propagated tops with very mild symptoms over the Brazilian sour-orange rootstocks being the sources upon which the aphids were fed to pick up, if possible, a mild strain of the virus. The results obtained 4 and 5 months after inoculation of the healthy test plants are in table 1.

The virus associated with mild symptoms was transmitted by the aphids

to some of the test plants in each trial. This is of special interest; it shows that the mild symptoms were not related to the possible factor of bud mutation in the Barão sweet orange, and that there exists, instead, a mild strain of the tristeza virus. In figure 1, B, visible differences in test-plant reactions 5 months after inoculation are tristeza symptoms (classes 4 and 3) in plants at left (s), resulting from inoculation with the severe virus strain, and mild symptoms in plants at right (m). In figure 1, C, the plant with mild symptoms (m) is stunted and shorter than the noninoculated healthy plant (n), but its growth is appreciably better than that of the severely diseased plant (s).

OTHER PLANT SELECTIONS WITH MILD TRISTEZA SYMPTOMS

Because of the one exceptional plant producing apparently healthy top growth from a Barão B virus-carrying bud over a nontolerant rootstock, it seemed desirable to examine again all of the records and the growth of all Barão sweet-orange tops in the large rootstock test. In this test there were

TABLE 2.—*Perpetuation of mild and severe forms of tristeza by budding*

Characteristics of selections from rootstock tests[a]			Symptoms[b], after 6 months on shoots from buds of selected plants on five Brazilian sour-orange rootstocks				
Bud origin	Sour-orange rootstocks	Top symptoms	1	2	3	4	5
Symptomless, virus-carrying Barão B orchard trees	Spain	Mild	0	m	0	–	0
	Egyptian	Mild	0	0	0	0	1
	Bitter Sweet (Stow)	Mild	m	m	m	0	0
	Dummitt	Mild	m	m	1	0	m
	Brazilian	Severe	2	2	3	3	3
Barão A seedlings	Algiers Seville	Mild	0	0	0	0	0
	Dummitt Bitter Sweet	Mild	0	0	m	m	m
	Bitter Sweet (Stow)	Mild	0	m	0	0	0
	Brazilian	Severe	2	3	3	3	3
Control	Seedlings of Barão	None	0	0	0	0	0

[a] Plants in the rootstock test were inoculated at least three times with viruliferous aphids; selections were made on the basis of mild and severe tristeza symptoms on the Barão sweet-orange tops over the various sour-orange rootstocks listed. The control buds were obtained from healthy, screenhouse-grown seedlings of Barão sweet orange.

[b] 0, apparently healthy; m, mild symptoms; 1 through 5, grades from definite initial to severe symptoms; –, tree died from undetermined cause.

not only the tops from the Barão B virus-carrying buds but also those from the Barão A buds from healthy screenhouse-grown seedlings, which, as tops in the rootstock test, had been repeatedly inoculated by means of viruliferous aphids. Out of 375 combinations of Barão sweet-orange tops over various nontolerant rootstocks, seven selections were made that had mild symptoms and a tendency toward recovery. Four of the selections had the Barão B virus-carrying bud origin and three were from Barão A originally healthy buds subsequently inoculated by means of viruliferous aphids. Five buds from each of these selections were budded over nontolerant Brazilian sour-

orange rootstocks. For purposes of comparison, Brazilian sour-orange rootstock plants were also budded with Barão A and B buds obtained from plants over sour-orange rootstock having severe tristeza symptoms. A control series of buddings was also included, consisting of buds from the Barão A healthy seedlings maintained in the screenhouse. The record of disease symptoms on all of the Barão tops 6 months after budding on the Brazilian sour-orange rootstock is in table 2.

All of the seven selections made from Barão sweet-orange tops with mild symptoms over various nontolerant rootstocks were, 6 months after budding over nontolerant Brazilian sour-orange rootstock, still apparently healthy or with only mild symptoms of tristeza disease. Buds selected from the severely diseased plants had definite symptoms of tristeza in their growth over this sour-orange rootstock. The healthy noninoculated control plants have remained healthy.

The results of this test indicated that there may be one or more mild tristeza virus strains: that one way of recognizing and selecting such strains may be by means of periodic disease records of the behavior of virus-infected citrus variety tops grown over a rootstock, such as sour orange, that is nontolerant of the tristeza virus; and further, that the mild form of the tristeza disease not only can be transmitted by buds, but also, in the case of the selections originating from the Barão A healthy buds, had been transmitted to these by means of the field collections of viruliferous aphids used to infest the plants. Again, the results suggest that the presence of the mild virus form had protected the plant selections and prevented them showing the most severe type of tristeza disease symptoms.

ADDITIONAL TEST OF TRANSFER OF STRAINS

Tests were made of the transmissibility of the mild and severe virus strains from Barão sweet-orange buds to healthy Valencia sweet-orange buds on Brazilian sour-orange rootstocks. Thirty Brazilian sour-orange seedling plants were budded first with one bud each from a virus-free Valencia sweet-orange plant. Then on ten of the sour-orange plants a second bud from the original Barão selection with mild tristeza symptoms was budded above the healthy Valencia bud. On another group of ten plants a second bud from a Barão selection with severe tristeza symptoms was budded above the healthy Valencia bud. The remaining 10 plants carried only the healthy Valencia sweet-orange buds.

After 1 month the sour-orange tops were cut back. Even 9 months from date of budding, not all of the Valencia buds had sprouted; but many did grow and the development of symptoms on these and on the Barão bud growth are summarized in table 3.

Shoots from the Barão sweet-orange buds selected from plants with severe disease symptoms developed severe symptoms, whereas those from buds from plants with only mild symptoms generally developed comparatively mild symptoms. It is evident that the tristeza virus passed from

the Barão top growth with severe symptoms to the Valencia branch below and caused typical tristeza disease symptoms. The similar pattern of behavior in the case of the Barão branches with mild symptoms and the occurrence of very mild symptoms or none on the Valencia branch suggest that the causal viruses are similar and that they differ chiefly in degree of symptom expression in the infected plants.

TABLE 3.—*Tristeza symptoms on Barão and Valencia sweet-orange shoots developed from buds on the same Brazilian sour-orange rootstock, in tests in which the Valencia buds were healthy, while the Barão buds came from plants with mild or severe symptoms of tristeza*

Row No.	Tristeza symptoms and bud source		Tristeza symptoms[a] on 10 shoots from inserted buds 9 months after budding on sour-orange rootstocks									
	Upper bud, Barão	Lower bud, Valencia	1	2	3	4	5	6	7	8	9	10
1	Mild		0	m	0	m	2	m	m	m	1	1
		Healthy	0	0	0	m	–	0	–	m	–	–
2	Severe		3	3	4	2	3	3	4	3	2	3
		Healthy	3	4	4	–	2	3	2	2	2	2
3		Healthy	0	0	0	0	0	0	0	0	0	0

[a] 0, apparently healthy; m, very mild symptoms; 1 through 5, grades from definite initial to severe symptoms. The minus signs indicate plants on which the inserted buds did not grow.

DISCUSSION

The fact that the mild form of the tristeza disease was transmitted both by means of buds and by the aphid vector shows that the causal agent is a virus. It is of special interest, however, that in the original plant selection and in the seven later selections some evidence was obtained that the plants with mild symptoms did not develop severe symptoms even though infested with aphids known to be carrying the severe strain of the virus. This protective action strongly indicates that the viruses involved are strains of the same virus. The observed comparative reactions of plants with mild and severe forms of the disease also indicate that the differences lie chiefly in degree of symptom expression, thus further indicating that the causal agent of the mild form is a strain of the tristeza virus.

The mild strain of the virus may be characterized as causing stunting and yellowing, and sometimes symptoms similar to those of zinc deficiency, in the sweet-orange tops budded over nontolerant sour-orange rootstocks. Occasionally plants with the mild form of the disease reached classes 1 and 2, i.e., initial symptoms of the severe type (Tables 2 and 3); such plants also appear to branch excessively and flower early, but have a greater tendency toward recovery from symptom expression than plants with the severe form.

Recognition of a mild form of tristeza constitutes a definite advance in our knowledge of this and similar diseases. As suggested by Bennett and

Costa (1), the differences in similar disorders on citrus in various parts of the world may be related to differences in virus strains introduced or developed in the respective areas.

It is certain that the finding of a mild strain of the tristeza virus opens up new fields for investigation. The fact that differences in the observed reactions of the mild and severe strains relate to time and degree rather than kind of symptom expression suggests the possibility that such disorders as quick decline of citrus or diseases of similar type may be caused by strains of this virus. Further knowledge of the reactions of the mild strain on grapefruit may lead to an even better understanding of the differences or similarities between the tristeza disease and quick decline of sweet oranges. It is known that some rootstocks, such as Nippon kumquat and *Severinia buxifolia,* appear to be more sensitive than sour orange to the tristeza virus; such rootstocks may have a special value in testing for and detecting the presence of the mild tristeza virus. Nothing is yet known of the distribution or prevalence of the mild strain or of its possible dominance, or ability to replace the severe strain or protect infected plants from showing severe symptoms. Studies by Kunkel (5) of peach viruses suggest the possibility that such procedures as cutting back and top-working of infected trees with buds carrying a very mild virus strain might lead to results of practical importance.

The mild form of the tristeza disease as obtained is not sufficiently mild to allow good development of infested sweet-orange tops over nontolerant rootstocks. On the other hand, it would be of interest to learn whether sweet-orange tops infected with the mild virus strain over tolerant rootstocks would grow and produce more or better fruit than comparable plants infected with the severe tristeza virus. These and other such studies resulting in the finding of even milder strains of the tristeza virus could conceivably lead to knowledge that might have a profound effect on nursery practices, selection of sources of budwood, quarantine measures, and general field practices.

SUMMARY

The data presented, giving observed plant responses in the tests described, show that a severe and a comparatively mild form of the tristeza disease have been transmitted by means of buds and the aphid vector.

Initial evidence is presented indicating that plants invaded by the mild form of the disease were protected against the severe form even though the plants were infested several times with viruliferous aphids. The differences between the mild and severe forms are in degree of symptom expression and in time required to show symptoms rather than in kind. These findings indicate that the virus causing mild tristeza symptoms is a strain of the tristeza virus.

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THE EFFICACY OF CERTAIN CHEMICAL SOIL TREATMENTS AGAINST MEADOW NEMATODES[1]

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While the efficacy of chemical soil fumigants in reducing root injury by meadow nematodes, *Pratylenchus* spp., has not been critically evaluated, several materials used against root-knot nematodes have also shown promise against meadow nematodes (1, 2, 3, 5, 7, 9). In North Carolina in 1948 counts of meadow nematodes made in connection with soil treatment tests previously reported (4) strongly indicated differences in efficacy of certain soil fumigants applied at comparable rates. Maximum meadow-nematode counts from 5-gm. root samples from tomato growing on plots treated with 600 lb. per acre of ethylene dibromide (10 per cent by volume), dichloropropene-dichloropropane mixture, or chloropicrin were 140, 4, and 4, respectively. At 200 lb. per acre, counts were 136, 124, and 68, respectively. These data were not considered conclusive but were deemed sufficiently indicative to warrant further investigation. The results of greenhouse tests with soil fumigants and of a field test with urea are herein reported.

MATERIALS AND METHODS

The soil treatment materials used in these tests were supplied by the manufacturers.[2] Three greenhouse tests were carried out in 10-in. clay pots filled with Norfolk sandy-loam soil obtained from the immediate vicinity of corn roots affected by meadow nematodes in field plots. The soil was screened through 1-cm.-mesh wire sieve before use. Fumigants were injected 5 in. deep into the center of the pots by means of a pipette. Rates of application were equivalent to 200, 400, and 600 lb. per acre. Soil temperatures at time of treatment were 68°, 78°, and 79° F. for tests A, B, and C, respectively. Two weeks after treatment, 40 gm. of commercial 6–8–6 fertilizer were applied to each pot and Golden Cross Bantam sweet corn was seeded. After emergence plants were thinned to three per pot. Three replicates of each treatment and the control were used in each test.

A modification of the method used by Graham (6) for determination of the relative number of meadow nematodes in plant roots was used. Ninety days after emergence the corn plants were removed from the pots and the root systems washed free of soil particles. Five 5-gm. root samples from each pot were finely chopped with scissors and put into Petri dishes con-

[1] Contribution from the Plant Pathology Section, Department of Botany, North Carolina Agricultural Experiment Station. Published with the approval of the Director as Paper No. 341 of the Journal Series.

[2] Chloropicrin by Innis, Speiden & Company; dichloropropene-dichloropropane mixture by the Shell Chemical Corporation; ethylene dibromide (10 per cent by volume) by Innis, Speiden & Company (ISCO) and by the Dow Chemical Company (Dow); and urea (Uramon) by the Ammonia Department, E. L. DuPont de Nemours & Company.

taining 30 cc. of tap water. From urea-treated field plots one composite sample consisting of tomato roots from each plant within each plot was taken after harvest. From this a 5-gm. sample was taken and treated as

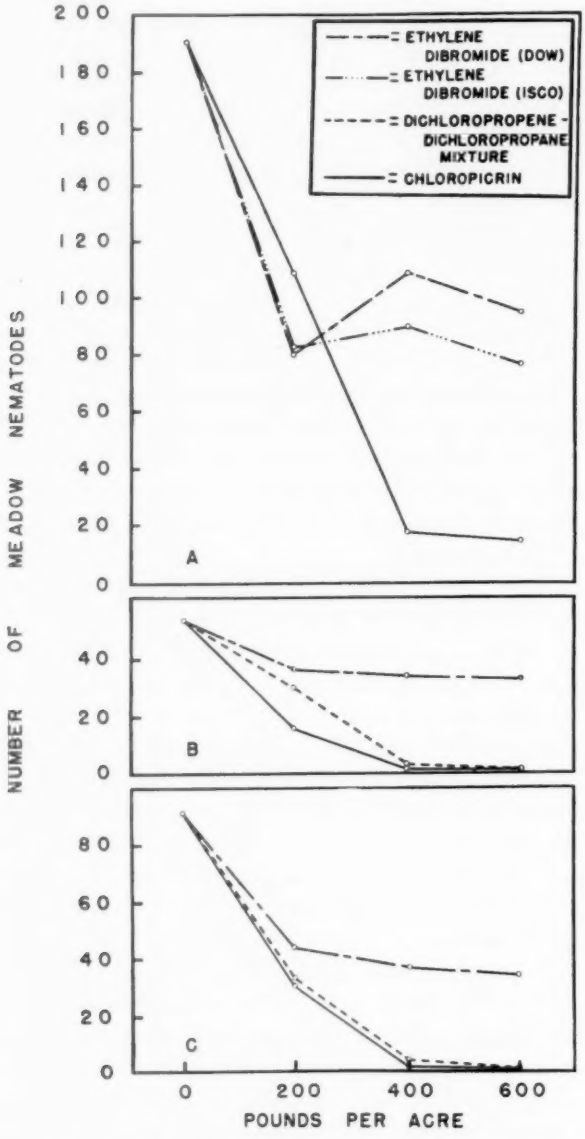

FIG. 1. Effect of soil treatment with volatile fumigants on the relative number of meadow nematodes in corn roots after 90 days.

above. All samples were held at about 25° C. for 48 to 72 hr.; then the root material was placed on a fine sieve which permitted passage of nematodes. This cleared the suspension of coarse debris and made counting

easier. Meadow nematodes were counted in each of 20 different fields, using a dissecting microscope equipped with 9 × oculars and a 4 × objective.

Three species of *Pratylenchus* were encountered in these tests; namely, *P. leiocephalus* Steiner (8), and two species as yet not formally described. While all three species were associated with lesions typical of meadow-nematode injury, their relative susceptibility to the various fumigants or their relative importance as root parasites was not determined.

Data were analyzed by the Department of Experimental Statistics, North Carolina State College. Only statistically significant results are stressed.

RESULTS

In comparison with the nontreated controls, all treatments in all greenhouse tests resulted in a highly significant reduction in the number of meadow nematodes (Fig. 1). Dichloropropene-dichloropropane mixture and chloropicrin applied at rates of 400 or 600 lb. per acre were more effective than at 200 lb. per acre. Ethylene dibromide was no more effective

TABLE 1.—*Effects of urea (Uramon) plus certain supplements upon the number of meadow nematodes occurring in tomato roots in field plots in the first year after treatment*

Treatment: Materials and rates (lb. per sq. yd.)	Average number of meadow nematodes from 5-gm. root sample
Uramon, ½	8.0
Do ½	1.2
Do ½ + peanut hull meal, 1	1.6
Do ½ + do	12.0
Do ½ + cottonseed meal, ½	4.0
Nontreated (check)	58.2
L. S. D. (0.05)	9.2

at 400 or 600 lb. per acre than at the 200-lb. rate in tests A and B, but in test C it was more effective at 600 lb. than at 200 lb. At 200 lb. per acre the two ethylene dibromide fumigants and chloropicrin were about equally effective in test A, but in tests B and C dichloropropene-dichloropropane mixture and chloropicrin were more effective in reducing the number of meadow nematodes than was ethylene dibromide.

In 1948, tomatoes were grown in field plots which had been treated with urea alone, or with urea plus cottonseed meal or peanut-hull meal, in the previous year. Four replicate plots 12 × 24 ft. each were treated in March 1947, and again October 1, 1947, with Uramon. Peanut-hull and cottonseed meal supplements were added October 30, 1947, by hand and disked 5 to 6 in. deep with tractor-drawn disks. Commercial 6–8–6 fertilizer (1200 lb./acre) was applied to all plots before Marglobe tomato plants were set on May 5, 1948, and nematode counts were made after fruit harvest in August. All treatments significantly reduced the number of meadow nematodes, in comparison with the nontreated control (Table 1). In an-

other experiment, data for which are not presented, it was found that meadow nematodes were as numerous in 1948 on plots treated with urea at 1 lb. per sq. yd. in 1945 as on nontreated plots.

SUMMARY

Tests with three soil fumigants showed that although treatment at the rate of 200 lb. per acre markedly reduced the number of meadow nematodes, survival was sufficient to result in moderate infestation of corn roots within 90 days. However, at rates of 400 and 600 lb. per acre, dichloropropene-dichloropropane mixture and chloropicrin were much more effective than at the 200 lb. rate and, at these higher rates, reduced the number of meadow nematodes to a much lower level than did the highest rate of ethylene dibromide.

Field tests indicated that treatments with urea alone and in combination with cottonseed meal or peanut-hull meal several months prior to the setting of tomato plants reduced the number of meadow nematodes to a very low level. However, those treatments neither eradicated the nematodes nor prevented rapid build-up in numbers by the third year after treatment.

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EFFECT OF AIR TEMPERATURE ON INCIDENCE AND DEVELOPMENT OF THE EARLY BLIGHT DISEASE OF TOMATO[1]

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The early blight disease of tomato (*Lycopersicon esculentum* Mill.) incited by *Alternaria solani* (E. & M.) Jones & Grout is seemingly coexistent with tomato culture, occurring throughout the United States and in foreign countries as well. Ordinarily, the disease does not become destructive in Wisconsin and other areas of similar climate until midseason or later. Various factors have been found to be related to early-blight severity and to account, in part, for its appearance after midseason. Thus, Horsfall and Heuberger (3) showed that plants increase in susceptibility as they mature, and when they become loaded with fruit. Barratt and Richards (2) reported a direct correlation between defoliation and physiological maturity of the plant. Horsfall and Heuberger (3) found early blight to be more severe on plants growing under low nitrogen nutrition, and Thomas (7) also reported that host nutrition affected disease severity. This paper is a report of studies to ascertain what effects, if any, air temperatures have on incidence and development of the disease.

The source and cultural type of strains of *Alternaria solani* used were as follows: Strain A, obtained from Dr. Curt Leben,[3] was highly chromogenic but nonsporulating; strain B, obtained from Dr. Lorin Richards,[4] was nonchromogenic but sporulating; strain C, isolated from a tomato leaf collected by Dr. L. J. Alexander, and strain D, obtained from Dr. C. F. Andrus, were nonchromogenic and nonsporulating.

Bonny Best tomato plants were used throughout the experiments. In defoliation studies they were grown singly in 4-in. clay pots in greenhouse compost and usually were inoculated when 8 to 10 in. high. Inoculum of isolate B, and of isolate A for some experiments, consisted of a suspension of conidia. Other inocula were prepared by growing the fungi in modified Czapek's solution[5] for 3 to 4 weeks and macerating the fungus mat in a Waring Blendor. Inocula were applied to leaves with an atomizer attached to a compressed air line. Plants were kept in a chamber at 100 per cent relative humidity for 24 to 48 hr. immediately after inoculation and then placed on greenhouse benches. Humidity chambers were placed in each of

[1] Published with approval of the Director of the Wisconsin Agricultural Experiment Station.

[2] Associate Professor of Plant Pathology, University of Wisconsin. Appreciation is expressed to Eugene Herrling for making the photographs used herein, and to Robert D. Raabe for assisting with some of the inoculations.

[3] Originally obtained from Dr. S. E. A. McCallan.

[4] Originally obtained from Dr. H. Rex Thomas.

[5] $NaNO_3$, 2 gm.; K_2HPO_4, 1 gm.; $MgSO_4$, 0.5 gm.; KCl, 0.5 gm.; $FeSO_4$, 0.01 gm.; dextrose, 30 gm.; distilled water to make 1 liter.

four houses which had controlled air temperatures of 16°, 20°, 24°, and 28° C. In each house, the temperature in the moist chamber was 2 to 3 degrees lower than the air temperatures, or approximately 13°, 17°, 21°, and 25°, respectively. Plants were taken from each moist chamber and divided equally among the four air temperatures. In each experiment, there were three plants at each air temperature from each moist chamber.

In studying the collar-rot phase, inoculations were made according to the method described by Andrus et al. (1). Immediately after transplanting into flats, inoculated plants were kept at the four air temperatures. Soil temperatures ranged about 2° lower than the respective air temperatures.

In calculating disease indices, five classes based on infection and development—none, trace, slight, moderate, and severe—were assigned values of 0, 10, 40, 70, and 100, respectively. In defoliation experiments the index given

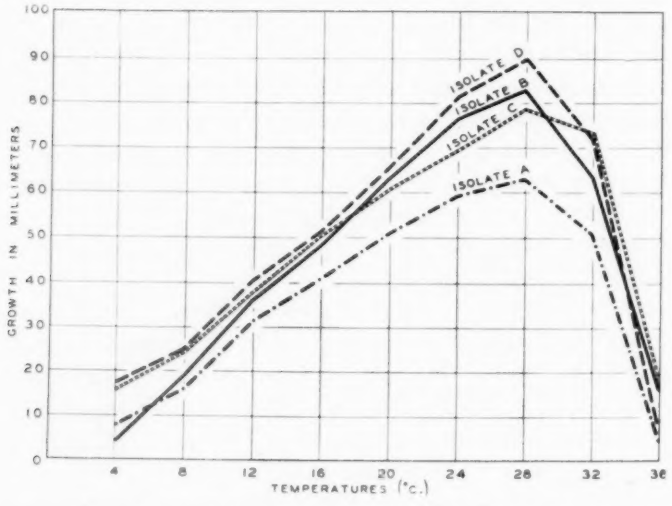

FIG. 1. Growth of four isolates of *Alternaria solani* after 6 days on potato-dextrose agar at various temperatures. Values shown are averages of five hypha-tip cultures of each isolate.

is the average value of three plants. Each plant value was obtained by rating three or four individual basal leaves, in order as they occurred on the plant. In the collar-rot experiments, the index was calculated by multiplying the number of plants in each class by the class value and dividing the sum of the products by the total number of plants.

EFFECT OF TEMPERATURE ON SPORE GERMINATION AND GROWTH OF ALTERNARIA SOLANI IN CULTURE

All four isolates of *Alternaria solani* were grown comparatively on the same batch of potato-dextrose agar at air temperatures of 4°, 8°, 12°, 16°, 20°, 24°, 28°, 32°, and 36° C. In three separate trials it was found (Fig. 1) that each isolate grew well over a wide range of temperature and each had approximately the same optimum temperature of 28° C. Growth at 24°

and 32° was almost as good as at 28° C.; no isolate grew well at 4° and 36°. Isolate A generally produced less growth than the other three. Pigment production by isolate A increased with rise in temperature, to a deep plum-red at high temperatures. Isolates C and D were very similar in cultural type, C having a bluish-green mycelium, and D being bluish-gray. Isolate B was black, due to profuse production of conidia.

Spore germination of isolates A, B, and C was rather uniform over a wide range of temperatures, 4° to 36° C. Spores of isolate A after 48 hr. had germinated at all temperatures, but at 4°, 32°, and 36° C. germ tubes were very short and could not have initiated infection. Isolates B and C resembled A in germination except that none occurred at 4° and only slight germination at 8° C. At 36° C. germ tubes were produced rapidly but soon rounded up and showed plasmolysis.

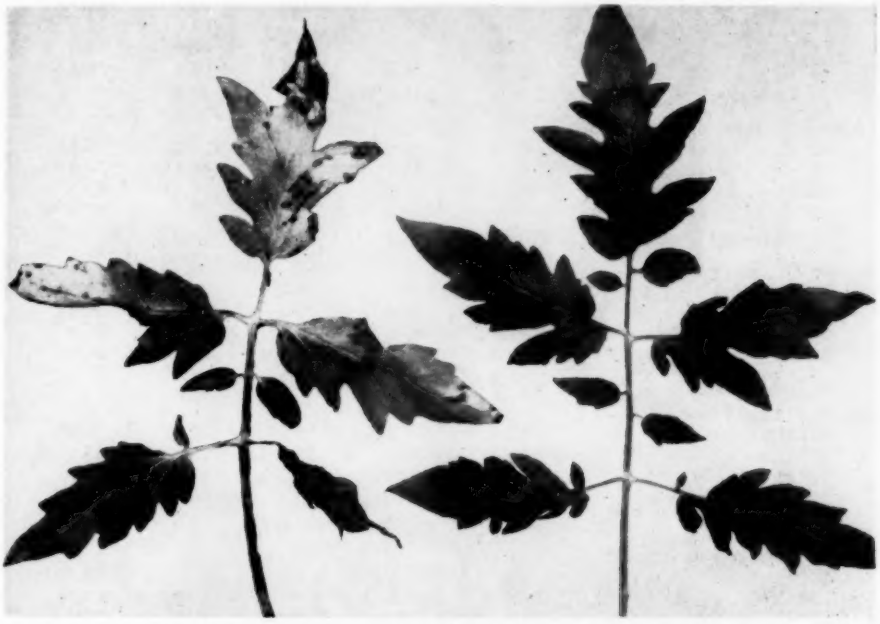

FIG. 2. Effect of air temperature on development of tomato early blight. Plants were inoculated with isolate A and incubated for 48 hr. at 25° C., then for 14 days at 16° C. (left) and 28° C. (right). Note severe development at 16° and only slight flecking at 28°.

EFFECT OF AIR TEMPERATURE ON LEAF INFECTION AND DEFOLIATION

Studies were conducted over a 3-year period and each experiment was repeated several times. Representative data for isolates A, C, and D are in table 1. With isolate A, temperature during the period plants were in the moist chamber had little effect on the amount of infection, but there was usually less at 25° and more at 17° C. The air temperatures at which plants were incubated following the moist-chamber period had pronounced effects upon disease development whether the inoculum was light or heavy. A

gradient of disease development occurred, very slight at 28° and very severe at 16° C. Lesions on plants placed at 28° C. rarely developed beyond a

TABLE 1.—*Effect of air temperature on infection and disease development in tomato when inoculated with isolates A, C, and D of Alternaria solani*

Type of inoculum, and moist-chamber temperatures[a]	Index of leaf spotting and defoliation at air temperature shown				Average
	16° C.	20° C.	24° C.	28° C.	
Isolate A: light inoculum[b]					
13° C.	40.0	23.3	13.3	10.0	21.7[d]
17° C.	60.0	20.0	10.0	10.0	25.0
21° C.	63.3	23.3	13.3	10.0	27.5
25° C.	60.0	23.3	10.0	10.0	25.8
Average[d]	55.8	22.5	11.7	10.0	
Isolate A: heavy inoculum[c]					
13° C.	60.0	40.0	23.0	10.0	33.3[e]
17° C.	70.0	57.0	27.0	10.0	41.0
21° C.	63.0	43.0	23.0	10.0	34.0
25° C.	50.0	30.0	23.3	10.0	28.3
Average[e]	60.8	42.5	23.3	10.0	
Isolate C: light inoculum[b]					
13° C.	30.0	20.0	15.0	10.0	18.8[d]
17° C.	60.0	43.3	23.3	18.3	36.2
21° C.	60.0	36.6	30.0	15.0	35.4
25° C.	54.4	43.8	33.3	16.7	37.1
Average[d]	51.1	35.9	25.4	15.0	
Isolate C: heavy inoculum[c]					
13° C.	30.0	36.7	26.7	11.7	26.3[e]
17° C.	60.0	46.7	70.0	63.3	60.0
21° C.	56.7	63.3	67.7	60.0	61.9
25° C.	70.0	53.3	63.3	70.0	64.2
Average[e]	54.2	50.0	56.9	51.3	
Isolate D: light inoculum[b]					
13° C.	10.0	7.8	10.0	10.0	9.5[d]
17° C.	80.0	43.3	56.7	40.0	55.0
21° C.	90.0	90.0	66.7	80.0	81.7
25° C.	100.0	80.0	93.3	40.0	78.3
Average[d]	70.0	55.3	56.7	42.5	
Isolate D: heavy inoculum[c]					
13° C.	10.0	10.0	10.0	10.0	10.0[e]
17° C.	66.6	60.0	70.0	80.0	69.2
21° C.	70.0	63.3	80.0	80.0	73.3
25° C.	70.0	63.3	50.0	56.6	60.0
Average[e]	54.2	49.2	52.5	56.7	

[a] Plants were kept in moist chambers for 48 hr. after inoculation and then divided equally between the air temperatures.

[b] Inoculum dilute: notes for isolate A taken 20 days after inoculation; for isolate C, 17 days; and for isolate D, 18 days after inoculation.

[c] Inoculum concentrated; notes for all isolates taken 14 days after inoculation.

[d] L.S.D. (19: 1) to compare any two averages for any one treatment: 5.9 for isolate A; 4.9 for isolate C; 7.2 for isolate D.

[e] L.S.D. (19: 1) to compare any two averages for any one treatment: 4.7 for isolate A; 5.1 for isolate C; 7.0 for isolate D.

fleck stage and ultimately plants were almost symptomless. At 16° C., however, lesions enlarged and caused severe defoliation (Fig. 2).

Isolates C and D were very similar in cultural characters, the chief dif-

ference being that sporulation was much easier to induce with ultraviolet irradiation in isolate C than in isolate D. These two isolates, and also isolate B, were very similar in the disease they produced. Infections with isolates C and D were suppressed markedly in the 13° moist chamber, especially for isolate D. In several experiments only occasional lesions developed on plants placed in the 13° moist chamber during the infection period. Isolate

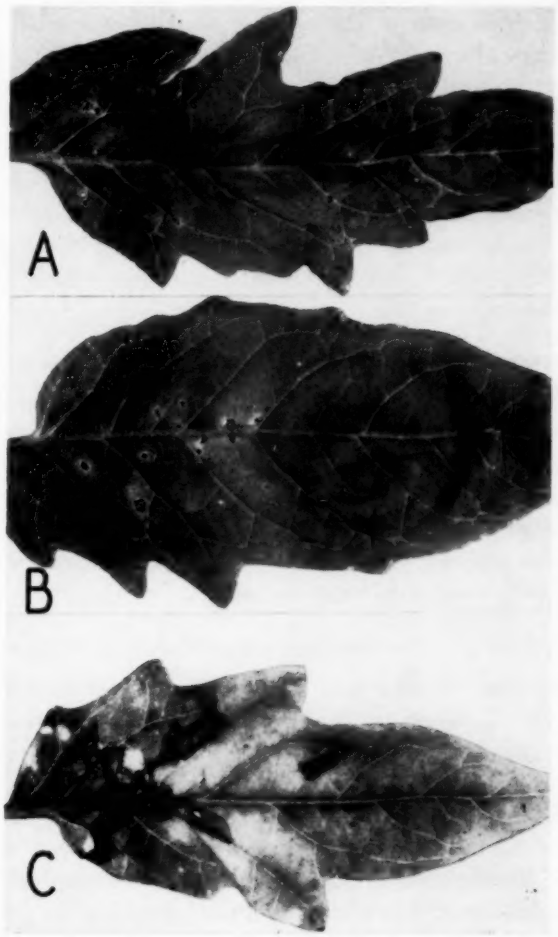

FIG. 3. Comparative development of intercostal leaf lesions produced on tomato when inoculated with strain B of *Alternaria solani* and incubated for 48 hr. at 21° C. and then for 14 days at 28° C. (A), 24° C. (B), and 16° C. (C).

B caused more infection at 13° C. than did isolates C and D. When relatively dilute inoculum was used, all three isolates had a gradient of disease development similar to that for isolate A, development being much more severe at 16° than at 28° C. When concentrated inoculum was used, however, there was little or no difference in disease development at the two air temperatures with any isolate; defoliation resulted before temperature

effects were apparent. The belief that production of a toxic agent by the fungus accounted for this rapid defoliation will be dealt with in a subsequent paper. More lesions occurred on vascular tissue (midrib, petioles, etc.), and the toxic substance spread rapidly. It was observed repeatedly that infections in intercostal areas did not develop into extensive lesions at 28°, but did so at 16° C. (Fig. 3), and the resulting defoliation was as rapid as from vascular lesions. Many vascular lesions did develop at 28° C., usually causing defoliation. The concentrated inoculum simply increased the number of vascular lesions. Isolate A apparently produced less of the toxic substance than did other isolates and this may account for its different development with concentrated inoculum at 28° C.

With all isolates, it was noticed that the lowest leaves on a plant were consistently more susceptible than younger leaves and abscised more quickly. Infections were obtained readily on the youngest leaves but lesion development was slow. Leaf lesions never assumed the target-board pattern typical of field infections. They were more nearly circular at higher temperatures than at low temperatures.

EFFECT OF PREINOCULATION TEMPERATURE ON INFECTION AND DEVELOPMENT

Two experiments were run in which plants were grown at 28° and 16° C. for 3 weeks and then inoculated with isolates A and D as described in the previous section. The plants had marked temperature growth effects at the time of inoculation. The results (not given) showed that the preinoculation treatment at high and low temperatures had no effect on the amount of infection and the severity of disease development with either strain. Data obtained were very similar to those in table 1.

EFFECT OF AIR TEMPERATURE ON SEVERITY OF COLLAR ROT

Each of the four isolates was tested in collar-rot inoculations at 16°, 20°, 24°, and 28° C., using the method of inoculation described by Andrus et al. (1). In most of the experiments plants were transplanted immediately after inoculation into soil which had been previously brought to the temperature desired. In some experiments duplicate trials were made in soil held at 22° C. air temperature for 48 hr. after inoculation and then placed at the four temperatures being studied. This specific post-inoculation treatment did not yield results different from those in which inoculated plants were placed directly at the temperatures being studied.

All four isolates showed a similar response to temperature (Table 2 and Fig. 4). A gradient of severity existed increasing from high to low temperature. Isolates C and D caused very shallow and small cankers which were definitely localized at 28° C. Isolate A and, to some extent, isolate B produced extensive infection at 28° C. but it was extremely superficial and caused little cessation of growth. At lower temperatures all isolates produced lesions which usually extended through the stem, causing severe stunting or death of the plant.

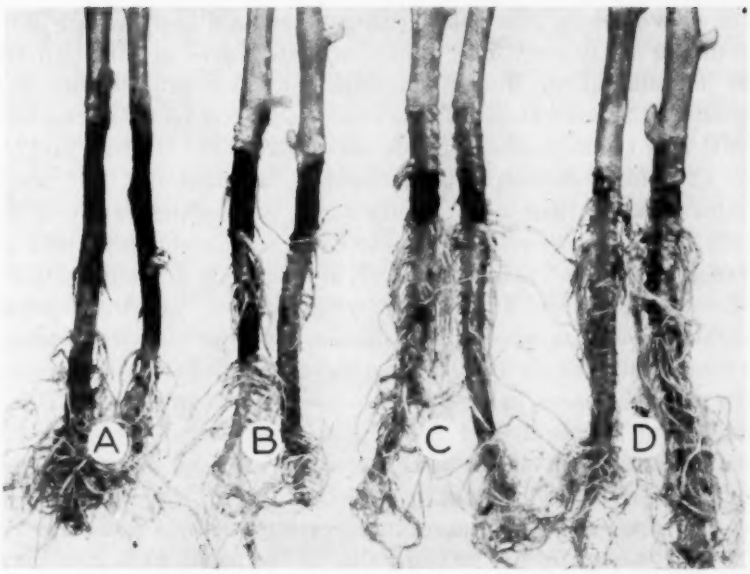

FIG. 4. Effect of air temperature on collar-rot severity with isolate D of *Alternaria solani*. A–D, plants grown for 4 weeks after inoculation at 16°, 20°, 24°, and 28° C., respectively.

TABLE 2.—*The effect of air temperature on the severity of the collar-rot phase of tomato early blight*

Isolate of *Alternaria solani*	Disease index at air temperature shown[a]			
	16° C.	20° C.	24° C.	28° C.
A	83.3[b]	56.5	40.9	30.8
B	90.4	80.1	62.8	38.8
C	80.3	60.6	49.8	23.9
D	67.5	43.3	18.1	12.3

[a] Temperature of soil in the flats ran approximately 2° lower than the air temperature.

[b] Values given are averages of five trials for isolate A, four trials for isolate D, three trials for isolate C, and only one trial for isolate B.

DISCUSSION

Defoliation of tomatoes by *Alternaria solani* is apparently influenced by several factors. The rate of defoliation is definitely related to the physiological maturity of the host. When several varieties of tomatoes of varying maturity dates are planted together and exposed to the same environment, the peak of defoliation of each is generally related to the peak fruit load of each. Aside from fruit set, there have been many observations to the effect that age of tissue affects the magnitude of severity of early blight. This is borne out by the present study in which it was found that the disease was generally most severe on the oldest leaves. Host nutrition, too, has been shown to affect the severity of early blight. Several cultural practices have been shown to be related to the amount of collar-rot infection (6).

Data presented in this paper indicate that air temperature to which tomato plants are exposed may influence both degree of infection and the severity of subsequent disease development. Air temperature did not greatly affect the amount of infection with isolate A of *A. solani*, but with isolates C and D much less infection occurred at 13° C. than at 17°, 21°, and 25° C. This reduction in the amount of infection at 13° C. was much greater for isolate D than for C; otherwise the two isolates were very similar. After the infection process was complete, disease development with all isolates was greater at 16° than at 20°, 24°, and 28° C. A gradient of severity existed, increasing from high to low temperatures. However, when very concentrated inoculum was applied, disease development with isolates B, C, and D was equally severe at all temperatures. Defoliation occurred before temperature differences in disease response were manifested. This rapid defoliation under concentrated inoculum was due to more extensive infection of vascular tissue than under light inoculum. It was observed repeatedly that infection loci on midrib or petiole tissue at 28° C. resulted in much quicker and much more defoliation than comparable infection on intercostal tissue. This is interpreted as being due to the production of a toxic substance which was readily transported through vascular tissue and which in itself effected defoliation. The failure of isolate A to develop extensively at 28° C. even on vascular tissue may be due either to inhibition of parasitic development or reduced production of the toxic material.

The collar-rot phase of the disease showed the same response to temperature as the leaf-spot phase. With all isolates, disease development was very severe at 16° and very slight at 28° C. Extensive infection occurred at 28° C. with isolate A, but lesions were very shallow and development was not severe enough to cause stunting. Kreutzer and Durrell (4), in reporting the collar-rot phase of the disease in Colorado, made no critical temperature study of the disease but stated that "the lower temperatures of spring are more favorable to the disease and less favorable to the growth of the tomato."

Moore (5) showed that under favorable humidity, tomato seedlings developed more leaf lesions at 74°–82° F. than at 54°–62° F. This is in agreement with results obtained herein with isolates C and D of *A. solani*. He made no mention of the effect of temperature on lesion development after infection.

SUMMARY

Four isolates of *Alternaria solani* (E. & M.) Jones & Grout have been studied comparatively in the laboratory and greenhouse. All isolates grew well on laboratory media over a wide range of temperatures, with optimum growth at 24°–28° C., and spores germinated over a range of 8° to 32° C.

In greenhouse studies, leaf infection with two isolates was markedly lower when plants were placed in humidity chambers of 13° and 17° C. for 48 hr. than when placed at 21° and 25° C. With the other two isolates considerable infection occurred at all four temperatures.

After the infection process was complete, disease development with all

isolates was much greater at 16° than at 28° C., when relatively dilute inoculum was used. A gradient of severity existed, increasing from 28° to 16° C. When relatively concentrated inoculum was used, however, defoliation with isolates B, C, and D was equally severe at all temperatures. This was due to more extensive infection of vascular tissue. Intercostal leaf lesions rarely developed to any extent with any isolate at 28° C.

A similar effect of temperature was found in studying the collar-rot phase of the disease. All four isolates produced progressively more severe symptoms from high to low temperature.

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INDUCED MODIFICATION OF SYMPTOM SEVERITY IN LITTLE CHERRY[1]

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(Accepted for publication September 28, 1950)

Symptoms of the virus disease little cherry, as the disease is recognized in the Kootenay region of British Columbia, are confined strictly to ripening fruits.[2] The symptoms include reduction in size, weight, and soluble-solids content of the fruits, modification of normal fruit coloration, and alteration of fruit shape.

The severity of little-cherry symptoms varies markedly from district to district, from tree to tree, from season to season in the individual tree, and frequently among the fruits of the individual tree in a single year. This evidence that the symptoms are labile has encouraged studies of the factors involved in symptom manifestation.

In 1947, the writers' attention was directed by E. C. Hunt, Supervising Horticulturist, to a diseased Lambert tree in the Nelson district, with one limb growing beneath a veranda roof at the north side of a farm home. This limb of the tree alone bore apparently normal fruits. In 1948, an adjacent limb, trained under the veranda early in the spring, produced during the same season fruits which were larger than those borne on the remainder of the tree.

In June, 1948, the greater portion of a single large limb of a diseased Lambert tree at Kootenay Bay was enclosed within a wooden shelter. This tree had a history of unusually consistent symptom severity. Incident light reaching the extremities of the limb on cloudy-bright midsummer days was reduced by this shelter to approximately 3 per cent. This shelter remained over the branch during the remainder of the 1948 season and the entire 1949 season.

The 1948 crop, which ripened about 5 weeks after erection of the shelter, was unaffected.

In 1949, the fruits of this tree were segregated into three groups, those borne on the sheltered portion of the sheltered limb, those on the unsheltered portion of the same limb, and those on unsheltered limbs (Fig. 1, A). Table 1 lists the diameters, weights, and soluble-solids content of the fruits from these three sources. The size, weight, shape, and coloration of fruits from the sheltered portion of the limb corresponded with those of normal Lambert fruits. The soluble-solids content was increased significantly, although it remained lower than in normal fruits.

Sheltered leaves and leaves from unsheltered limbs were subjected to tests by the technique recommended by Lindner[3] for demonstration of stone-

[1] Contribution No. 1024 from Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Foster, W. R., and T. B. Lott. "Little cherry," a virus disease. Sci. Agr. **27**: 1–6. 1947.

[3] Lindner, R. C. A rapid chemical test for some plant virus diseases. Science **107**: 17–19. 1948.

fruit virus diseases. All tests of unsheltered leaves yielded positive results, with light to dark red solutions, whereas negative results were given consistently by sheltered leaves.

As this experiment involved modifications of the three factors, light, temperature, and humidity, and subjection of the foliage as well as the fruits to these altered conditions, no final conclusions can be drawn about the significance of the individual factors, or the means by which their effects are exerted. However, the modifications of temperature and humidity were

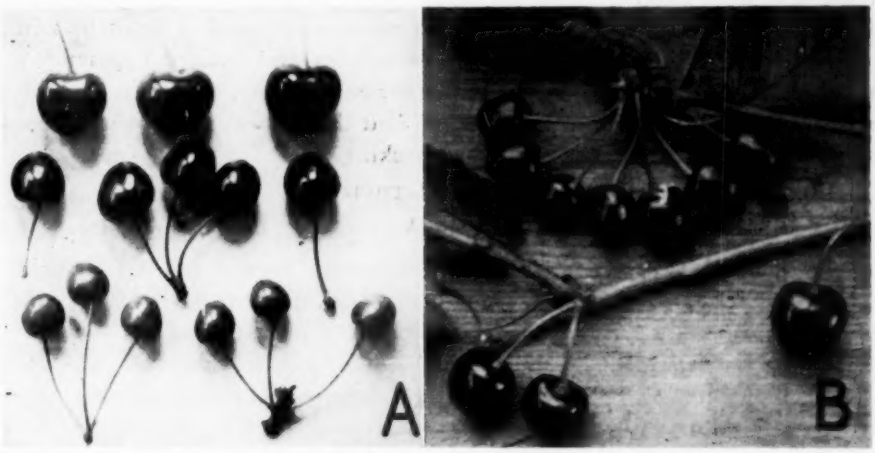

FIG. 1, A. Modification of little-cherry symptoms by the sheltering of a diseased limb. Upper row, Lambert fruits from sheltered portion of sheltered limb. Middle row, fruits from unsheltered portion of same limb. Lower row, fruits with severe symptoms from unsheltered limbs. B. Relation of foliage abundance to symptom masking. Upper: typical little-cherry symptoms on leafed spur. Lower: partial symptom masking in fruits of sparsely-leafed branch.

TABLE 1.—*Modification of little-cherry fruit symptoms by the sheltering of a single limb of a diseased tree*

Source of fruits	Number of fruits	Average diameter (mm.)	Range of diameters (mm.)	Average weight (gm.)	Average soluble solids
Unsheltered limbs	544	20.1	16–27	5.1	8.7
Unsheltered portion of sheltered limb	9	24.6	22–26	7.0	10.1
Sheltered portion of sheltered limb	30	28.2	26–32	9.5	11.3

slight, whereas the reduction of light under the shelter was very severe, causing partial etiolation and abnormal elongation of the leaves. Moreover, the indications of partial masking of symptoms in fruits borne on unshaded portions of the shaded branch suggest that the severity of symptoms in fruits on a given limb may be a manifestation of the conditions imposed on the bulk of the foliage of that limb.

Evidence of a relation between abundance of foliage and severity of fruit symptoms has been provided by observations made during three seasons in

diseased orchards. The browsing of lower limbs of diseased trees by deer early in the seasons of 1947 and 1948, and the stripping of buds by the grouse population in certain districts during the severe 1948–1949 winter, resulted in the production of fruits on spurs or lengths of branch which were almost or entirely devoid of leaves. Invariably a large proportion of the fruits borne under these conditions have been much larger than the fruits produced in close proximity to heavy foliage (Fig. 1, B). A similar result is obtained when heel-spur budding is employed for the experimental transmission of little cherry. Usually the transferred spur produces one or more fruits without leaves, and invariably symptoms are masked in such fruits.

Information gained from these observations and limited experimental results is being used as the basis for further experiments, designed to resolve more specifically the responsible factors and the critical adjustments necessary for varying degrees of symptom masking.

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NEW PHYSIOLOGIC RACES OF USTILAGO NIGRA FROM THE UNITED STATES AND ISRAEL[1]

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(Accepted for publication October 10, 1950)

Since its discovery in 1932 (5, 6) *Ustilago nigra* Tapke, the cause of the black or nigra loose smut of barley, has been collected in this country in 23 States (7). It is also widespread in Canada and Turkey (1, 2, 3). In 1944 Minz (4) reported that 11 of 31 collections of barley loose smut in Israel were *U. nigra*. Upon request, Minz sent 16 collections of *U. nigra* to the writer in 1945. These were increased in the greenhouse during the winter of 1945–1946, and then used in the study of physiologic races reported here.

Table 1 shows the reactions of eight differential varieties of spring barley to 13 physiologic races of *U. nigra*, nine from the United States and four from the Israel collections. The first seven of the nine races from the United States have been described previously and their reactions on six differential barley varieties under field conditions at Ithaca, N. Y., reported (7). The reactions shown in table 1 were obtained in greenhouse tests at Beltsville, Maryland, in 1947 and 1948. The testing of the Israel collections in the greenhouse from September to early December precluded the likelihood of disseminating the new races to barley fields. The tests also served to compare reactions of the Israel races with those of the United States races under identical conditions, and to compare the greenhouse reactions of the nine United States races with those obtained in the field at Ithaca, New York, in 1947.

The differential hosts used in the present study were pure lines of the eight spring barleys (Table 1). The seed used in both years of the test had been produced at Aberdeen, Idaho, in 1946. In other respects the methods and materials were the same as those employed in previous studies of physiologic races in *U. nigra* (7) and *U. hordei* (8).

Consistent differences in the pathogenicity of the 13 physiologic races were demonstrated (Table 1). Field infections with the nine races from the United States were lighter than those obtained in the greenhouse, but the results of this and similar previous field tests (7) have been adequate to delineate each race clearly. The greenhouse experiments indicated a low susceptibility in Trebi to U. S. races 1 and 3 that was not evident in the field test.

The 16 Israel collections comprised six distinct races. Five collections were similar to U. S. race 4, and six were similar to U. S. race 6. The remaining five collections comprised four new races. One of these was allotted to race 10, one to race 11, one to race 12, and two to race 13. Three of the four new Israel races were able to infect Pannier, although no collection of

[1] Cooperative investigations of the U. S. Department of Agriculture and the New York (Cornell) Agricultural Experiment Station.

TABLE 1.—*Percentages of loose smut produced in eight differential varieties of spring barley by 18 physiological races of Ustilago nigra from the United States or Israel*

Smut source, and race No.	Location of test, and year[a]	Percentages of smutted heads in								Race identified by the susceptibility of	Number of times race collected in the United States
		Excelsior C.I. 1248	Hannchen C.I. 531	Himalaya C.I. 1312	Lion C.I. 923	Nepal C.I. 595	Odessa C.I. 934	Pannier C.I. 1330	Trebi C.I. 936		
U. S.											
1	F 1947	0	0	0	0	0	44	0	0	Odessa and Trebi (light infection in Trebi)	40
	GH 1947	0	0	0	0	0	100	0	12		
	GH 1948	0	0	0	0	0	100	0	12		
2	F 1947	0	30	0	0	0	56	0	18	Hannchen, Odessa, and Trebi	15
	GH 1947	0	86	0	0	0	100	0	92		
	GH 1948	0	95	0	0	0	95	0	67		
3	F 1947	0	0	0	16	0	72	0	0	Lion, Odessa, and Trebi (light infection in Trebi)	2
	GH 1947	0	0	0	57	0	100	0	15		
	GH 1948	0	0	0	52	0	100	0	19		
4	F 1947	0	47	0	28	0	75	0	42	Hannchen, Lion, Odessa, and Trebi	86
	GH 1947	0	96	0	55	0	100	0	92		
	GH 1948	0	93	0	54	0	100	0	91		
5	F 1947	0	31	56	14	71	72	0	46	Hannchen, Himalaya, Lion, Nepal, Odessa, and Trebi	7
	GH 1947	0	96	74	34	100	100	0	83		
	GH 1948	0	100	94	53	100	96	0	94		
6	F 1947	60	23	43	17	60	69	0	48	All varieties except Pannier	15
	GH 1947	100	82	81	60	100	100	0	94		
	GH 1948	90	100	76	53	100	100	0	92		
7	F 1947	0	24	37	0	40	60	0	24	Hannchen, Himalaya, Nepal, Odessa, and Trebi	3
	GH 1947	0	67	86	0	100	100	0	93		
	GH 1948	0	88	100	0	100	97	0	97		
8	F 1947	8	0	0	2	46	60	0	32	Excelsior, Lion, Nepal, Odessa, and Trebi (light infection in Excelsior)	1
	GH 1947	14	0	0	20	50	100	0	88		
	GH 1948	9	0	0	26	80	100	0	86		
9	F 1947	5	0	0	0	13	45	0	0	Excelsior, Nepal, and Odessa (light infection in Excelsior)	1
	GH 1947	9	0	0	0	26	100	0	0		
	GH 1948	8	0	0	0	30	100	0	0		
Israel											
10	GH 1947	0	85	0	42	0	100	0	0	Hannchen, Lion, and Odessa	0
	GH 1948	0	96	0	42	0	100	0	0		
11	GH 1947	0	15	0	50	0	100	37	19	Hannchen, Lion, Odessa, Pannier, and Trebi (light infection in Trebi)	0
	GH 1948	0	23	0	27	0	100	55	13		
12	GH 1947	74	53	0	50	100	100	13	26	All varieties except Himalaya	0
	GH 1948	100	36	0	28	100	100	11	29		
13	GH 1947	68	70	67	60	78	100	43	22	All varieties	0
	GH 1948	91	96	88	71	90	100	38	19		

[a] F indicates field test at Ithaca, N. Y., in 1947; GH indicates greenhouse tests at Beltsville, Md., in 1947 or 1948.

U. nigra from the United States thus far tested has been able to cause smut in this variety. Race 13, one of the Israel isolates, is the only one so far tested that is able to produce smut in all of the eight differential varieties. It is evident, therefore, that barley seed from Israel may harbor dangerous races of the pathogen.

The new U. S. races 8 and 9 were collected in Missouri and Ohio, respectively. To date each has been found but once.

SUMMARY

The black or nigra loose smut of barley (*Ustilago nigra* Tapke), now widely distributed in the United States, Canada, and Turkey, was reported in 11 of 31 collections of barley loose smut in Israel in 1944.

Six physiologic races were isolated from 16 Israel collections sent to the United States. Four of these races do not occur, or at least have not yet been found, in the United States. All of the tests were conducted in a greenhouse during the early winter to guard against possible escape of the pathogen.

Three of the four new races from Israel produced smut in Pannier. This variety has been immune from all of the nine physiologic races of *U. nigra* collected in the United States to date.

The reactions of eight differential varieties of barley grown in the greenhouse to 13 races of *U. nigra*, nine from the United States and four from Israel, are presented.

In a comparative study, field infections with the nine United States races were lighter than those in the greenhouse and the latter tests brought out in Trebi a low susceptibility to several races not evident in the field. Results of the present and similar previous field tests, however, have been adequate to clearly delineate each race.

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ULTRAVIOLET ABSORPTION AS A MEASURE OF TOBACCO-MOSAIC VIRUS NUCLEOPROTEIN

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(Accepted for publication October 3, 1950)

The concentration of unpurified or purified tobacco-mosaic virus preparations has been frequently determined by the local-lesion method of Holmes (7) and Samuel and Bald (12), a method based on infectivity. This is, however, strictly a measure of active virus. Where appreciable quantities of noninfective virus nucleoprotein are found, as reported by Bawden and Pirie (3), Sigurgeirsson and Stanley (13), and Takahashi and Rawlins (17), it is apparent that a true measure of total virus nucleoprotein is not provided by this method. Moreover, where excessive aggregation of virus particles has been brought about, the concentration indicated by lesion count is often lower than the actual concentration (14).

If, for example, the formation of total virus protein in tissue is to be followed, a method is desired that does not fail to reveal the noninfective and the true concentration of highly aggregated virus. Several published methods which account for the total virus protein have been based on the known physical and chemical properties of the virus.

Beale and Lojkin (5), in their use of the precipitin reaction as a basis for a quantitative method, took advantage of the direct proportionality between the antigenic properties of tobacco-mosaic virus and its infectivity. More recently Bawden and Pirie (2, 3, 4) have made effective use of this method.

The critical dilution of stream double refraction, a physical method, which depends on the asymmetry or rod shape of tobacco-mosaic virus particles, has been used by Takahashi and Rawlins (15, 16) to show differences in virus concentration. The method was modified by Lauffer and Stanley (8), who used a photoelectric cell to measure the intensity of stream double refraction. They found that, per unit concentration of tobacco-mosaic virus, the stream double refraction varied from preparation to preparation depending upon modifications of preparative technic. As the pH of the solution was changed from 7 to 5 the double refraction increased markedly. It appears that this double refraction was a function of length of particle since end-to-end aggregation is known to take place at the lower pH (14).

Electron microscopy has been employed by Backus and Williams (1) to assay quantitatively a specimen having one or more components. Polystyrene latex spheres of determined concentration were used as a reference component.

The foregoing four methods do not require purified virus preparations and hence may be applied to crude preparations.

Purified virus suspensions may be assayed by several methods. If suf-

ficient material is available the virus may be dried and then weighed. Usually the Kjeldahl nitrogen of a suspension is determined, and, since tobacco-mosaic virus contains 16.6 per cent nitrogen, the Kjeldahl value is converted to protein by multiplying by the factor of 6.06.

The intensity of the characteristic blue color developed by the Folin phenol reagent (6) with three amino acid constituents of tobacco-mosaic virus, tyrosine, tryptophane, and cysteine, has been measured by Lojkin and Beale (10) by means of a photoelectric colorimeter. Microquantities of virus ranging from 0.006 to 0.6 mg. have been determined by this method.

Estimation of other amino acid constituents of tobacco-mosaic virus protein that occur in high proportion and for which satisfactory color reactions are known, such as arginine and the Sakaguchi (11) reaction (intense red with free guanidine), might also be developed into a quantitative method.

The chemical properties of phosphoric acid, purine and pyrimidine bases, and d-ribose, constituents of the nucleic acid moiety of the virus protein, may also be utilized in determining virus concentration. The current methods of determining both phosphate (phosphomolybdic acid) and ribose (orcinol) involve chemical reactions that not only consume time but are somewhat laborious for use in a facile routine manner. This is equally true of the Kjeldahl nitrogen and the phenol and guanidine reactions. On the other hand, the concentration of the purine and pyrimidine components, which are characterized by high ultraviolet absorption, may be estimated directly with a spectrophotometer without preliminary chemical or physical treatments.

A characteristic ultraviolet absorption maximum of purified tobacco-mosaic virus protein was shown by Lavin and Stanley (9) to occur at 2650 Å. It seems that if reasonable accuracy and reproducibility are attainable this distinctive absorption could be used as a basis for a simple and satisfactory method of virus assay.

Tobacco-mosaic virus was prepared from 20-day inoculated tobacco leaves by two cycles of alternate low- and high-speed centrifugations. At all times the pH was kept at pH 7.0 by means of 0.1 molal phosphate buffer. The final pellet was suspended in 0.01 M phosphate buffer at pH 7.0. Three small aliquots were taken, their Kjeldahl nitrogen determined, and the values converted to weight of virus protein. A series of known dilutions were made and their absorbancy (negative $\log^{10}$ of transmission) determined by means of a Beckman model DU spectrophotometer set for wave length 265 mμ. The absorbancy plotted against mg. of virus nucleoprotein per ml. yields a straight line (Fig. 1) indicating that Beer's law is applicable. The absorbancy of a purified virus suspension of unknown concentration or of various dilutions of the suspensions may therefore be read directly in the spectrophotometer and the reading converted to mg. of virus nucleoprotein per ml. from the calibration curve. Extracts of healthy tobacco leaf tissue similarly purified have given negligible readings. In

practice this method is rapid and simple and has given reproducible results.

To summarize, ultraviolet (265 mμ) absorbancy has been utilized as a basis for a rapid and simple method of determining tobacco-mosaic virus

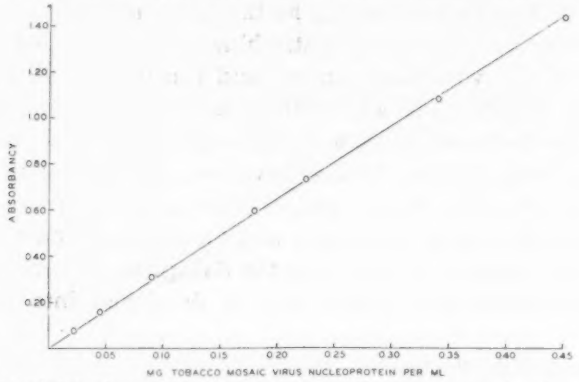

FIG. 1. Ultraviolet absorption as a measure of tobacco-mosaic virus nucleoprotein. Abscissae are mg. of virus nucleoprotein per ml. as calculated from nitrogen determinations; the ordinates their absorbancy at wave length 265 mμ. The straight line plot shows that a regular relation exists between dilution of virus nucleoprotein and absorbancy.

concentration. The method will account for the noninfective and aggregated virus nucleoprotein as well as the active unit virus.

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EFFECTS OF A SOURCE OF HEAVY INFECTION ON THE DEVELOPMENT OF DUTCH ELM DISEASE IN A COMMUNITY

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(Accepted for publication October 16, 1950)

When the responsibility for the control of the Dutch elm disease (*Ceratostomella ulmi*) was placed on property owners and local governments, they needed information on what would happen to elms in communities where infected trees were permitted to produce large numbers of bark beetles. A study plot was therefore established in a residential area in Essex County, New Jersey.

The plot, 1 mile in diameter, was bisected by a small valley. Native and planted elms, 2600 elms per sq. mi. in the valley and 1700 per sq. mi. on the higher land, constituted about 25 per cent of the tree population. Most of the street elms were large, whereas the natural ones were of all sizes. Most of the trees received limited care and were healthy.

At the center of the plot was a large-crowned, 40-in. elm, infected in 1934 and killed by the Dutch elm disease in 1944. During 1943 and early 1944, thousands of bark beetles, *Scolytus multistriatus* (Marsh), developed in the tree, but no other important source of bark beetles were within or at the borders of the plot in early 1944. The disease and bark-beetle carriers had been known in the area since 1933, but until 1943 they had been suppressed by a general control program.

The nature and values of property in the plot and the interests of the property owners in their elms were varied. Three towns joined near the center of the plot, and a railroad and a public-service right-of-way crossed it. A small part of the plot was in a commercial zone where the street elms had limited room for root growth. Many of the homes were on small lots with an elm or two on the front or rear lawn. Some properties were large and well landscaped. Garden apartments and single houses were constructed on two vacant lots while the study was under way. Some owners considered all trees a nuisance, other owners were indifferent; some prized their elms highly and others merely wished to avoid the cost of removing dead elms.

STUDY METHOD

All elms 2 in. or more in diameter were plotted on a large-scale map. They were inspected in early summer and again in late summer in 1944, 1945, and 1946. Each elm with symptoms of Dutch elm disease was examined, and its location, size, disease development, and condition as to bark beetle were recorded. Samples from diseased parts were taken for laboratory culture to determine definitely the cause of the disease. On subse-

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quent visits the condition of the tree, with respect to symptoms and bark-beetle infestation, was recorded. In 1946 cursory inspections were made of the elms within a half-mile band around the study plot. After 1946 the elms in which there was some evidence of recovery from Dutch elm disease were observed yearly.

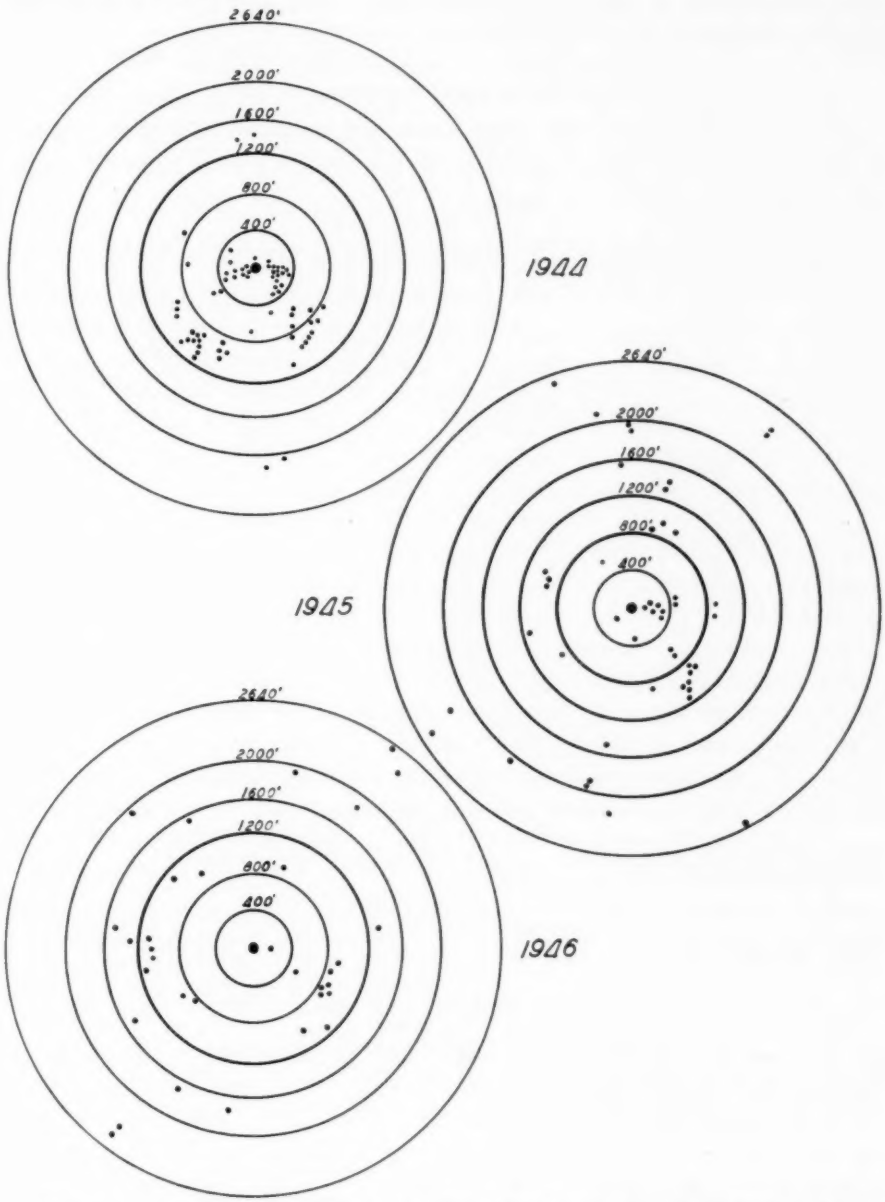

FIG. 1. Distribution of Dutch elm disease found in an Essex County, New Jersey, study plot in 1944, 1945, and 1946. Each dot represents one diseased elm found during the year indicated. Large center dot represents the source tree.

The State informed owners of the presence of known diseased trees on their properties, and some trees were removed before bark beetles could develop. The control work done in the study plot was nearly normal for such an area and did not reduce the value of the study. A concentration of brush elms near the center of the plot was cleared without regard to the Dutch elm disease in the winter of 1944–1945. The number of elms on this lot is not included in the data presented.

NUMBER AND LOCATION OF DISEASED TREES

The basic data developed at the time the plot was established and the numbers of diseased trees found for the three years are in table 1. Locations of diseased trees are in figure 1.

TABLE 1.—*Numbers of trees with Dutch elm disease in 1944, 1945, and 1946, located at varying distances from a 1943–1944 source of infection*

Zone No.	Zone limits, in feet	Zone area, acres	Number of elms in 1944	Number of diseased elms		
				1944	1945	1946
1	0–400	11.5	64	29	8	1
2	401–800	34.5	124	9	5	1
3	801–1200	58.0	372	27	18	17
4	1201–1600	80.0	262	2	4	5
5	1601–2000	104.0	237	0	4	5
6	2001–2640	215.0	294	2	9	4
	Total	503.0	1353	69	48	33

The number of diseased trees in the area more than 800 ft. from the source was about the same each year (31, 35, and 31 for the 3 years respectively). Disease symptoms appeared in about 3 per cent of the elms each year. In an area between $\frac{1}{2}$ and 1 mi. from the center, observed in 1946, about the same conditions existed as in outer zones of the study plot. Thus the 3 per cent yearly rate of infection appears to be a measure of the en-

TABLE 2.—*Weighted values of number of diseased elms (Table 1), showing effect of distance and time on disease incidence*

Zone No.	Zone limits, in feet	Relative amount of Dutch elm disease[a]		
		1944	1945	1946
1	0–400	66.6	33.6	5.4
2	401–800	10.7	6.4	1.3
3	801–1200	10.7	7.8	7.6
4	1201–1600	1.1	2.3	2.9
5	1601–2000	0.0	2.5	3.2
6	2001–2640	1.0	4.5	2.0

[a] $\dfrac{\text{Number of diseased elms}}{\text{Total number of elms minus diseased elms of previous year}} \times 147$

The factor 147 was established by converting to unity the 1944 figures for zone 6 (see table 1). Sample computation: 2 (diseased) : 294 (elms) = 0.0068; 1 ÷ 0.0068 = 147.

demic condition in the area during the 3-year study period. In contrast, the rate of infection in 1944 was 45 per cent in zone 1 and 7 per cent in zone 2. In 1945 it dropped to 23 per cent in zone 1 and 4 per cent in zone 2, and in 1946 to 4 per cent and 1 per cent respectively.

Although the numbers of elms per acre in zones 1 and 3 were higher and those in zone 6 were lower than the plot average, there was no apparent relation between the elm population and the intensity of the disease. With 1 representing the disease intensity in zone 6 in 1944, the relative intensity of disease in the other zones in the 3 years is shown in table 2. The effects of the disease source at the center of the plot diminished rapidly with distance and time.

BARK-BEETLE DEVELOPMENT

Plot records show that 30 of the trees having Dutch elm disease became generally infested with bark beetles in 1944 and 1945. Nine of these 30 trees had diameters of 2 to 4 in., nine were 5 to 10 in. in diameter, seven were 11 to 26 in., and five were 27 to 35 in.

These trees were scattered throughout the plot, and most of them were not destroyed in time to prevent emergence of bark beetles. However, by the end of 1946 no areas of high disease intensity had developed around any of these trees, and in 1945 and 1946 the amount of the disease over the area in general decreased compared with 1944.

Several factors may have contributed to this static condition, which was considerably different from that around the large central source tree from which many bark beetles emerged in 1943 and 1944. The 12 largest elms (11 to 35 in. in diameter), which became infested in 1944 and 1945, were not so heavily infested as was the source tree, and harbored proportionately fewer beetles. Because they were scattered, the total land area and the total number of elms within 400 ft. of all of them were much greater than around the one original tree. It is also very likely that the seasonal development of the elms and weather conditions during periods of bark-beetle abundance greatly affected the rate of infection each year.

In the study plot 87 other trees were diseased: some were only lightly infested, some were not sufficiently affected by Dutch elm disease to support an infestation, and some were removed before their condition could be studied.

EVIDENCES OF RECOVERY FROM THE DISEASE

As only a few diseased trees found in the study plot were removed, it was possible to follow the progress of the disease in 95 infected elms, of which 19 trees of various sizes had some disease resistance (Table 3).

All the known discoloration was removed from four of the 19 elms at the time of sampling, and they apparently were freed of the disease. One of them died in 1947, probably from a new infection. Three were pruned within a month to a year after the disease was first detected, but such

TABLE 3.—*Size of diseased trees, and number of trees with some disease resistance*

Diameter, inches	Number of trees	
	Diseased	Resistant
2 to 3	17	0
4 to 6	20	5
7 to 12	18	3
13 to 18	11	3
19 or more	29	8
Total	95	19

pruning probably was not a factor in the recovery of the trees. Sixteen of the trees had local symptoms only. One-tenth of the crown of one tree and one-fourth of the crown of another were dead the year after symptoms were noted, but both trees were symptomless thereafter. A 39-in. elm found in 1946 was discolored throughout the current water-conducting vessels but was only slightly wilted. No wilting was observed in this tree in 1947, but in 1949 symptoms of Dutch elm disease developed throughout its crown. Six of the elms that showed evidence of recovery were removed for reasons other than Dutch elm disease, and the apparently reinfected tree was removed. Of the 19 resistant trees, 12 are still standing and will be observed periodically in the future.

PRACTICAL PERSPECTIVE

The focal point of this investigation, a 40-in. diameter, large-crowned American elm, became symptomatic and was sampled and confirmed for Dutch elm disease in the summer of 1934. The owner of the property, who resided in Virginia, demurred on the felling, removal, and burning of this tree because of the prohibitive cost, $325. The shade-tree officials of the municipality in which this tree was located and those of the two practically contiguous municipalities were importuned to arrange for its removal, to eliminate this source of beetles and inoculum. No action was taken, however, and by the spring of 1944 tremendous numbers of bark beetles were emerging from the tree.

There is now no need for the recurrence of a situation such as that caused by the large central source tree, even though the tree is not felled, removed, and burned. The thorough spraying of such a tree with a 2 per cent DDT emulsion will trap emerging bark beetles before they can carry the inoculum to other trees. Unfortunately this information was not available in the spring of 1944, the critical time for the dissemination of the Dutch elm disease fungus from this large infected tree.

SUMMARY

Many near-by elm trees contracted the Dutch elm disease from a single large tree from which bark beetles were permitted to escape. The menace

of the source tree was largely spent at a distance of 800 ft. The failure of the disease to become epidemic over the entire plot by 1946 suggests that natural factors may prevent the development or perpetuation of the epidemic of Dutch elm disease in a locality.

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THE DISPERSAL OF VIABLE FUNGUS SPORES BY INSECTICIDES

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(Accepted for publication October 18, 1950)

Conidia of *Septoria obesa*, the causal agent of one of the chrysanthemum leaf-spot diseases, are extruded from pycnidia in a gelatinous matrix and cannot be removed readily except when wet. They are, therefore, not adapted for efficient dispersal by air currents and it has been generally accepted that localized intensification is dependent almost exclusively upon the splashing of raindrops or overhead irrigation water. In the fall of 1940, however, a commercial chrysanthemum grower, Harry L. Allyn of Big Flats, New York, suggested that viable spores of *S. obesa* might be dispersed in the spray blast accompanying the application of certain insecticides. The suggestion was made in an attempt to account for the occasional development of spots on foliage of field-grown plants above the zone normally affected by splashing raindrops. It appeared reasonable that spores of the pathogen might be picked up from cirri on pycnidia on the lower foliage by a nonfungicidal insecticide and carried to the healthy upper foliage, since the spray blast was directed upwards to obtain coverage of the lower leaf surfaces.

Shortly after this a case was seen which pointed very strongly to insecticidal sprays as agents of spore dispersal. In this instance the chrysanthemums were grown under glass and leaf spot had developed abundantly throughout the foliage to the tops of plants 3 ft. or more tall. The grower was certain that at no time had the plants been splashed with clear water, but stated that they had frequently been sprayed very thoroughly with a rotenone insecticide. The circumstances strongly indicated the insecticidal spray as the disseminating agent. Experiments, briefly reported elsewhere,[1] confirmed this hypothesis. These experiments, and others recently concluded on the dispersal of urediospores of snapdragon rust (*Puccinia antirrhini*) by insecticides in current usage, are here discussed in detail. Studies on spore dispersal by selective fungicides are reported in a separate paper.[2]

ROTENONE AND THIOCYANATE INSECTICIDES AND SEPTORIA LEAF SPOT OF CHRYSANTHEMUMS

An exploratory experiment was conducted in which spores of *S. obesa* were suspended in Righto (a rotenone concentrate), 1:400, and in clear water. Each suspension was then atomized with a sterilized atomizer onto

[1] Dimock, A. W. Relation of insecticides to the development of diseases of ornamentals. (Abstr.) Phytopath. 38: 313. 1948.

[2] Dimock, A. W. The dispersal of viable spores of phytopathogenic fungi by fungicidal sprays. Phytopath. 41: 157–163. 1951.

three Petri plates of acidified agar. After 3 or 4 days isolated spores could be seen germinating in abundance over the surfaces of the three plates sprayed with the clear water suspension, whereas only 10 or 12 germinating spores could be found on the three plates sprayed with Righto suspension. These spores continued to grow when transferred to tubes of agar. However, the percentage germination in Righto plates was extremely low.

In a companion experiment suspensions of spores in Righto, 1:400, and in clear water were each sprayed on the lower leaf surfaces of four potted, single-stem chrysanthemum plants (var. Yellow Fellow). The plants were immediately placed in an infection chamber for 48 hr. and then removed to the greenhouse bench. After 4 weeks, four spots had developed on the Righto-sprayed plants, and 211 on checks sprayed with clear water. This indicated that Righto might function to a very limited degree in the dispersal of spores of *S. obesa*.

TABLE 1.—*Germination of spores on agar and infection of chrysanthemum foliage following inoculation with conidia of Septoria obesa suspended in insecticidal spray preparations and clear water*

Material	Type[a]	Dilution	Germination on agar[b]	Number of leaves		Number of spots	
				Inoculated	Infected	Total	Per 10 leaves
Hitox	R	1:400	–	68	0	0	0.0
Lethane 440	T	1:300	–	79	8	9	1.1
Stantex R50	R	1:400	+	73	15	17	2.3
Loro	T	1:600	+	86	15	22	2.6
R. S. 380	R&T	1:400	+	67	18	20	3.0
Rotogreen (N.F.)	R	1:400	–	68	18	26	3.8
Red Arrow	R	1:400	–	73	23	37	5.1
Rotogreen 106	R&T	1:600	–	77	42	78	10.1
Formula 157	R	1:400	+	65	45	117	18.0
Water (check)			+	64	46	138	21.6

[a] R indicates rotenone, T indicates thiocyanate. The complete formulae of these concentrates were not made available. Most of them are no longer manufactured.

[b] + indicates germination; – indicates no germination.

A more extensive test employed nine insecticides which at the time were in common use in floricultural greenhouses. The same methods were used as in the previous tests. A single concentrated suspension of spores from pure culture was prepared in distilled water and used as the diluent for all materials in the Petri-plate tests, and a similar suspension of spores from sporulating leaf lesions was prepared in tap water and used as the diluent for all materials used in the plant inoculation tests. The inoculated plants were kept in the infection chamber for 4 days and then removed to the greenhouse bench. The results of this test (Table 1) provide evidence that viable conidia of *S. obesa* could be dispersed to a significant degree by many of the insecticidal sprays tested. Since the rotenone sprays all contained 1 to 2 per cent rotenone, the differences in infection are pre-

sumed to be due to differences in the carriers and emulsifying agents used in the concentrates. Attention is drawn to the fact that the laboratory tests on Petri plates did not give a reliable indication of performance in plant inoculation tests.

In another test using the same techniques but including two additional materials, Righto and NNOR, the results (Table 2) confirmed the evidence of the previous test, although the ranking of the materials varied somewhat. It is unfortunate that the number of inoculated leaves was not recorded in this test, since calculation of the number of spots per inoculated leaf would have given a better index of the performance of the materials. However, the first five materials in the first test were again the first five of that group of materials in the second test. Hitox again completely prevented infection and Righto showed high fungicidal value as it had in the exploratory test. In the companion laboratory tests none of the materials completely

TABLE 2.—*Infection of chrysanthemum foliage following inoculation with conidia of Septoria obesa suspended in insecticidal spray preparations and tap water*

Material	Type[a]	Dilution	Number of	
			Infected leaves	Spots
Hitox	R	1:400	0	0
Loro	T	1:600	1	1
Righto	R	1:400	1	1
Stantex R50	R	1:400	1	2
RS 380	R & T	1:400	3	5
Lethane 440	T	1:300	6	7
Formula 157	R	1:400	8	9
NNOR	R	1:400	10	11
Rotogreen 106	R & T	1:600	12	12
Red Arrow	R	1:400	16	19
Rotogreen (N.F.)	R	1:400	18	19
Water (check)	.....	.....	25	35

[a] R indicates rotenone, T indicates thiocyanate. The complete formulae of these concentrates were not made available. Most of them are no longer manufactured.

prevented growth on agar in Petri plates.

In a final experiment using the same techniques but employing only the two Rotogreen formulations and Righto, all at 1:400 dilution, and using five plants for each material, Righto was again highly effective, limiting infection to four spots on two leaves. With Rotogreen 106 and Rotogreen N.F., respectively, 38 and 57 infected leaves had 147 and 430 spots; in the check (water), 79 infected leaves had 1519 spots.

These experiments demonstrated conclusively that many of the insecticides tested had little fungicidal value against *Septoria obesa*. Employment of these materials as sprays for chrysanthemum foliage might, therefore, result in an intensification of Septoria leaf spot if primary infection was initially present and fungicidal sprays were not being employed. The use of materials possessing high fungicidal value, such as Hitox and Righto, would involve little or no hazard of disease intensification.

NEWER INSECTICIDES AND SNAPDRAGON RUST

A single experiment was made in the spring of 1950 to determine whether or not some of the more recently developed insecticides might function in the dispersal of viable urediospores of the snapdragon rust fungus (*Puccinia antirrhini*). Suspensions or emulsions of the insecticides were made up at the indicated concentrations, and copiously sporulating infected leaves were added to each preparation within 2 min. before use. The spores were suspended by vigorous shaking. It was considered impractical to standardize the spore concentration, but an attempt was made to use about the same amount of sporulating material for each preparation. The suspensions were atomized onto the lower leaf surfaces of three young snapdragon plants (*Antirrhinum majus*) in each case and the plants placed immediately in an infection chamber at 62° F. All plants were removed about 48 hr. later and placed on the greenhouse bench. Results were recorded 15 days after inoculation, prior to the rupture of any secondary uredia (Table 3).

TABLE 3.—*Infection of snapdragons resulting from inoculation with urediospores of Puccinia antirrhini suspended in insecticidal preparations*

Material, and source	Amount per 100 gal.	Number of leaves		Number of infections per 100 leaves
		Inoculated	Infected	
DDT, 50 per cent w.p. (Gesarol D.T. 50, Geigy)	2 lb.	92	7	17
Benzene hexachloride, 50 per cent w.p. (DuPont, 5 per cent gamma)	2 lb.	74	25	146
Parathion, 15 per cent w.p. (Thiophos 3422, Monsanto)	1.5 lb.	80	59	670
Tetraethyl pyrophosphate, 40 per cent emul. (Tetracide, Niagara)	½ pt.	86	68	755
Chlordane, 50 per cent w.p. (Synchlor, Naugatuck)	2 lb.	100	80	1027
Water (check)	.....	84	68	976

Although these results cannot be considered to have quantitative significance, it is obvious that the parathion, tetraethyl pyrophosphate, and chlordane preparations employed had little or no fungicidal value and could readily function in the dispersal of viable urediospores of *Puccinia antirrhini*. The benzene hexachloride and DDT preparations employed apparently had considerable fungicidal value against *P. antirrhini* and would be less effective in the dispersal of inoculum.

DISCUSSION

While it is generally recognized that most insecticides possess little or no fungicidal value, the rather obvious corollary that insecticidal sprays may function in the dissemination of spores of phytopathogenic fungi has apparently received little or no recognition. The experiments herein reported provide convincing evidence that such a possibility exists. Whether

or not insecticidal sprays contribute significantly to the intensification of diseases under field conditions is not certain. Their potential role in the dispersal of viable inoculum, however, should not be overlooked in the preparation of spray programs or in the development of insecticidal formulations.

SUMMARY

Chrysanthemum foliage was successfully inoculated with *Septoria obesa*, and snapdragon foliage with *Puccinia antirrhini*, by spraying the plants with suspensions of spores of the respective fungi in spraying-strength preparations of a number of insecticides. Many of the insecticidal formulations had no significant fungicidal value; others were moderately to highly fungicidal. The potential role of insecticidal sprays of low fungicidal value in the localized dispersal of viable spores of phytopathogenic fungi deserves recognition.

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THE DISPERSAL OF VIABLE SPORES OF PHYTOPATHOGENIC FUNGI BY FUNGICIDAL SPRAYS

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(Accepted for publication October 18, 1950)

In the course of examining spray test plots for snapdragon rust (*Puccinia antirrhini*) at Ithaca, New York, in the fall of 1949, it was noted that Bioquin 1 (80 per cent copper-8-quinolinolate) failed to give appreciable control of the disease. This in itself was not unanticipated, since other copper fungicides previously tested had failed to give adequate control, but closer inspection of the plants revealed a pattern of occurrence and a density of infection that seemed inconsistent with what might be expected from natural dissemination of urediospores by air currents or splashing raindrops. It was therefore conjectured that viable urediospores were actually being picked up in substantial numbers from uredia on the lower leaves and transported to the upper leaves by the spray stream itself, and further, that a proportion of such spores subsequently germinated and caused infections. Exploratory experiments designed to test this hypothesis are reported herein.

EXPERIMENTAL SPRAY PLOTS

The spray tests referred to above consisted of four 10-plant plots for each treatment, set out in randomized blocks on June 7 and inoculated with urediospore suspensions on July 6 and July 15. Spraying was started on June 13 and continued on a weekly schedule until September 8; a power sprayer at 250 lb. pressure was employed, and the spray was directed upward so as to cover the lower leaf surfaces. Final data were recorded September 15 (Table 1).

TABLE 1.—*Results of fungicidal spray tests for snapdragon rust control. Ithaca, New York, 1949*

Material	Concentration (lb./100 gal.)	Average[a] rust rating	Plants with[b] secondary fungi
Parzate	1.5	0.2	0
Fermate	1.5	1.0	4
CR305	1.5	2.1	34
SR406	1	2.4	23
Bioquin 1	1.5	2.9	1
Check		3.3	40

[a] 0, no infection; 1, few scattered infections; 2, infections all over plant but moderate in numbers; 3, plants heavily covered with infections; 4, plants dead.

[b] Total number of plants, of 40 possible, with rust infections invaded by secondary fungi.

It is significant that although Bioquin 1 failed to control rust it almost completely prevented invasion by secondary fungi (principally *Fusarium* sp.) which in the CR305, SR406, and check plots rapidly advanced and de-

stroyed the leaves.[1] This circumstance permitted observation of the density and pattern of distribution of primary infections in the Bioquin 1 plots. Had this fungicide not been selective in its action, the phenomenon here discussed would not have been detected. However, close scrutiny of a great many plants revealed large numbers of primary flecks on a high percentage of the young leaves near the tops of the shoots. Such a pattern, in the writer's opinion, could not have resulted from natural dissemination of urediospores under the prevailing conditions, and could be explained only on the assumption of forceful artificial dispersal of spores. The only possible agent of artificial dissemination in this instance appeared to be the spray blast itself. In theory, dry urediospores from uredia on the lower foliage and stems might be carried to the upper leaves by the air currents associated with the spray stream and might initiate infections during subsequent periods of rain or dew; or the spores might be picked up in the liquid phase and carried to the upper leaves, germinating either directly in the spray droplets or subsequently in rain or dew deposits. The possibility of dissemination in the liquid phase of fungicidal sprays was explored in the following experiments.

INOCULATION TESTS

Spore-fungicide suspensions. In the first test a concentrated suspension of spores was prepared, using as the liquid phase a suspension of copper-8-quinolinolate (Bioquin 1 at 1 lb./100 gal.). A comparable suspension was also made up in clear water. Accurate equalization of the spore concentrations appeared impractical, but an attempt was made to use approximately the same quantity of rust-infected material in preparing the two

TABLE 2.—*Primary infection of Puccinia antirrhini on snapdragon plants inoculated with spore-fungicide and spore-water suspensions*

Plant No.	Copper-8-quinolinolate		Water	
	Total infections	Average per leaf	Total infections	Average per leaf
1	166	9	302	15
2	68	3	412	22
3	106	5	770	45
4	118	5	424	24
5	120	7	506	25
Av.	116	6	483	26

suspensions. Five plants (var. Afterglow) were inoculated with each preparation by atomizing the suspensions on the lower leaf surfaces. The plants were then placed immediately in the saturated atmosphere of an infection chamber for 3 days, after which they were removed to the greenhouse bench. Eight days after inoculation numerous primary flecks were seen on the plants of both series, and final data (Table 2) were recorded

[1] Dimock, A. W., and K. F. Baker. Effect of climate on disease development, injuriousness, and fungicidal control, as exemplified by snapdragon rust. Phytopath. (In press.)

about a week later, just before rupture of secondary uredia.

These results demonstrate convincingly that urediospores of *P. antirrhini* may be effectively disseminated in splashed water, and hence, presumably, in any nonfungicidal spray. Furthermore, the evidence is clear that viable urediospores may be carried in sprays containing the selective fungicide copper-8-quinolinolate, and that they may germinate directly in the spray droplets and initiate new infections. It is also evident that this compound is somewhat fungicidal to the urediospores.

A second test was conducted in which urediospore suspensions were made employing preparations of the following materials as the liquid phase: Bioquin 1, Parzate (zinc ethylene bis dithiocarbamate), Fermate (ferric dimethyl dithiocarbamate), CR305 (bis (2-hydroxy,5-chlorophenyl) sulfide), Phygon XL (2, 3-dichloro,1, 4-naphthoquinone), SR406 (N-trichloro-methylthio-tetrahydrophthalimide), wettable sulfur, 4–4–100 Bordeaux mixture, and clear water. All fungicides except Bordeaux were used at 1 lb. per 100 gal., without spreader. The methods employed were the same as in the previous test. Temperature of the infection chamber for the 42-hr. infection period was approximately 63° F. Results were recorded on all series 11 days after inoculation, and supplementary counts were made 2 weeks later.

The results (Table 3) fully substantiate those of the previous test with

TABLE 3.—*Rust infections resulting from inoculating snapdragons with urediospore-water and urediospore-fungicide suspensions. Totals for five plants of each treatment; counts made October 28, 1949, except as noted*

Material	Number of leaves infected		Infections	
	Total	Variation among plants	Total	Average per inoculated leaf[a]
Water			8000[b]	33.1
Bioquin 1	227	40–50	1300[b]	5.6
CR305	42(56)[c]	5–15	45	.....
Bordeaux	23(42)	1–8	28	.....
SR406	22(33)	3–8	35	.....
Sulfur	19(23)	1–13	23	.....
Fermate	1(1)	0–1	1	.....
Phygon XL	0(0)	.....	0	.....
Parzate	0(0)	.....	0	.....

[a] Based on counts on ten leaves taken at random from the central portion of each of the five plants.

[b] Estimated.

[c] Figures in parentheses represent counts of primary infections made Nov. 11, 1949; leaves had been removed from Bioquin 1 plants in making first count.

regard to the ability of Bioquin 1 sprays to function in the intensification of snapdragon rust. Furthermore, the reduction in the amount of primary infection effected by the fungicide agrees reasonably well in the two tests.

The other fungicides fell into two groups. The first group, including Bordeaux mixture, CR305, SR406, and wettable sulfur, showed fairly high

fungicidal activity against the rust spores, but still could function to some extent in the dispersal of effective inoculum to new infection centers when used at the concentrations and under the conditions here employed. The second group, including Fermate, Phygon XL, and Parzate, completely or almost completely prevented infection and hence, as used in these tests, could not well function in the dispersal of effective inoculum.

It is of considerable interest that in this type of test the fungicides ranked in about the same order as in the conventional field tests reported earlier in this paper, in which the materials were applied as protectants. This relationship is true also of Bordeaux mixture, wettable sulfur, and Phygon, which in field tests at Ithaca have given consistently poor control, indifferent control, and excellent control, respectively.

Wettable sulfur-urediospore suspensions at low temperature. Since it has been shown[2, 3] that the fungicidal efficiency of sulfur is greatly reduced at temperatures as low as 50° F., and since this is in the optimum range for snapdragon-rust infection, it was reasoned that the use of wettable sulfur sprays at low temperatures might serve to disperse effective inoculum. To test this hypothesis, two experiments were conducted. In the first, three small snapdragon plants (Afterglow) which had been held at 50° F. and three held at 75° F. for 4–6 hr. were sprayed with urediospore-

TABLE 4.—*Infection of snapdragon plants with rust following inoculation with suspensions of spores and wettable sulfur at 50°, 70°, and 75° F.*

Temperature	Sulfur	Number of leaves		Infections		
		Inoculated	Infected	Actual	Per 100 leaves	Per cent of check
50° F.	Absent	18	17	1205	6694	.....
50° F.	Present	30	24	257	857	13.0
70° F.	Present	28	6	10	36	.....
75° F.	Absent	28	25	400	1429	.....
75° F.	Present	30	2	2	7	0.5

wettable sulfur (1 lb./100 gal.) suspensions in water at 50° and 75° F., respectively, and immediately covered with bell jars adjusted to these temperatures. The spore concentrations of the two suspensions were not equalized. After 42 hr. in these controlled-temperature chambers the bell jars were removed and the plants transferred to the greenhouse bench. Data were recorded 14 days later: at 50° F., 1551 infections developed on 66 of the 92 leaves inoculated. Infections per plant were 392, 494, and 665. At 75° F., only four infections developed on three of the 92 leaves inoculated.

In the second experiment, two plants and bell jars were held for 6 hr. at 50° and 75° F. and a single plant and jar at 70° F. One plant at 50° and one at 75° F. were inoculated with a concentrated suspension of uredio-

[2] Doran, W. L. Rust of Antirrhinum. Mass. Agr. Exp. Sta. Bul. 202: 39–66. 1921.
[3] Yarwood, C. E. Effect of temperature on the fungicidal action of sulphur. Phytopath. 40: 173–180. 1950.

spores in clear water, and the remaining three plants were inoculated with a urediospore-wettable sulfur (1 lb./100 gal.) suspension made up with the above spore suspension as the liquid phase. The spore concentration in this test was thus equalized, though it was impossible to equalize the deposit on the leaves. The bell jars were removed after 3 days and the plants transferred to the greenhouse bench. Data were recorded 10 days later (Table 4).

The results of these tests show that at temperatures as low as 50° F. wettable sulfur sprays could function in the dispersal of viable spores of the snapdragon-rust fungus. The effectiveness of such sprays in spore dispersal would probably be influenced by spore load, concentration of wettable sulfur, and particle size, as well as temperature. At 70°–75° F. or higher, wettable sulfur sprays should have little or no effect in spore dispersal.

DISCUSSION

Experimental evidence is herein provided that the employment of (a) an inadequate fungicide, or (b) a good fungicide under conditions unfavorable for its action, may actually serve to intensify rather than control a fungus disease. The writer has reported elsewhere[4, 5] evidence that the employment of certain insecticidal sprays similarly may serve to disperse effective inoculum of phytopathogenic fungi. This phenomenon has interesting implications.

On several occasions the writer has heard fungicide investigators state that the future of fungicide research lies in the development of specific materials for given diseases. Possibly this is true, but the evidence presented herein indicates that the employment of highly specific materials may involve a hitherto unrecognized hazard. It is evident from the data in table 1 that copper-8-quinolinolate is a highly selective material giving excellent control of Fusarium, yet failing to control, and apparently intensifying, the rust.

An intriguing corollary of the observed phenomenon is that the use of a selective fungicide for a given important disease may excellently control that disease, yet raise another and previously trivial disease to a position of importance. A hypothetical example would be the employment of copper-8-quinolinolate for the control of the important Alternaria blight (*A. dianthi* and *A. dianthicola*) of carnations in plantings where both Alternaria blight and rust (*Uromyces caryophyllinus*) were present. The latter disease is usually of minor importance on carnations, normally limited to the lower foliage under good cultural conditions. Copper-8-quinolinolate consistently proved superior to all other materials recently tested[6] for the control of Alternaria blight, but let us assume that it would influence carnation rust as it does snapdragon rust. It would follow, then, that the use

[4] Dimock, A. W. Relation of insecticides to the development of diseases of ornamentals. (Abstr.) Phytopath. 38: 313. 1948.

[5] Dimock, A. W. The dispersal of viable fungus spores by insecticides. Phytopath. 41: 152–156. 1951.

[6] Tests conducted by C. H. Ford during 1948 and 1949 at Farmingdale, N. Y.

of copper-8-quinolinolate in such a hypothetical case, while excellently controlling the blight pathogen, might actually intensify the rust.

A similar situation might result if lime-sulfur were employed for the control of Alternaria blight or rust of carnations in a planting in which bacterial leaf spot (*Pseudomonas woodsii*) was also present. Lime-sulfur provides reasonably good control of the first two diseases, but evidence was obtained by Dr. J. M. Bickerton in 1948[7] that carnations sprayed with this fungicide had highly significant increases in bacterial leaf spot over unsprayed check plants. These results might possibly be explained on the basis of dissemination of viable bacteria in droplets of a spray material of low bactericidal value under the prevailing conditions, although limited tests to prove this were unfruitful.

It is conceivable also that intensification of any disease caused by a freely sporulating fungus might result from the employment at reduced concentration of a fungicide normally highly effective at an adequate dosage rate. Expressed otherwise, for any given fungicide and any given fungus there may be a concentration of toxicant below which the number of viable spores carried by the spray blast would be sufficient to initiate new infections in appreciable numbers.

A further implication is that the employment of a fungicide under environmental conditions unfavorable for its action, yet favorable for germination of the fungus spores involved, might well result in intensification rather than control of the disease. It has been shown here that applications of wettable sulfur spray made during low temperature periods might serve to intensify rather than control snapdragon rust. Analogous situations may exist with other fungicides and other diseases.

SUMMARY

Experimental evidence is presented indicating that copper-8-quinolinolate sprays may not only fail to control snapdragon rust, but actually intensify it by carrying viable spores in the spray stream from sporulating uredia to new infection courts. Snapdragon plants were successfully inoculated with suspensions of urediospores in copper-8-quinolinolate spray, though the amount of infection averaged only 17 to 23 per cent of that obtained with spores suspended in clear water. Successful inoculation was also obtained with spores suspended in Bordeaux mixture, wettable sulfur spray, and two experimental fungicidal sprays, though the amount of infection was very low. Inoculations with spores suspended in Fermate, Phygon XL, and Parzate were unsuccessful.

Infection resulting from inoculation with urediospores suspended in wettable sulfur spray was abundant at 50° F., but only negligible at 70° to 75° F.

It is conjectured that intensification of infection may result from the use of (a) a fungicide of low inherent toxicity to the fungus spores con-

[7] Unpublished report on file with the author.

cerned, (b) a normally effective fungicide at too low a concentration, or (c) a normally effective fungicide under conditions unfavorable for its action.

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THE RELATIONSHIP BETWEEN FUNGICIDAL ACTIVITY AND CHEMICAL CONSTITUTION[1]

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The present work was initiated with two purposes: 1) to evaluate the fungicidal properties of readily available compounds, and 2) to point out whatever relationships may be present between fungicidal properties and chemical constitution. From a theoretical standpoint, the latter is particularly pertinent in view of conflicting observations found in the literature.

Although Klarmann, Gates, and Shternov (6) have reported that fungicides show specificity for certain organisms, Suter (14) and Jensen (4) indicate that, in general, a trend of fungicidal effectiveness in a series of compounds will tend to repeat itself with various organisms.

In view of the above, it is believed that an evaluation of the fungicidal properties of compounds can be made by testing them against one of the more resistant species of fungi, such as *Aspergillus niger*.

Keeping in mind the possible practical use of the fungicides, it was decided that a knowledge of the limiting concentration preventing growth of *A. niger* in a nutrient medium would be of more value than the phenol coefficients as determined by various methods.

EXPERIMENTAL PROCEDURES

The nutrient medium contained 8 gm. of yeast extract, 2 gm. peptone, 10 gm. glucose, and 500 ml. distilled water. To determine the optimum pH for growth, 50-ml. portions of the medium were pipetted into volumetric flasks and the pH was adjusted in the various flasks to values from 8.0 to 3.5 by the addition of acetic acid or NaOH. Volume was made up to 100 ml. with distilled water and the medium was autoclaved at 15 lb. for 20 min., then inoculated with a loopful of *A. niger* grown on an agar slant. A comparison of the number of spores contained in each flask after incubation at 37° C. for 48 hr. indicated that the organisms grew best at pH 6–7. In all subsequent inoculations, the medium was adjusted to pH 6–7 by the addition of dipotassium hydrogen phosphate.

Solutions of the inhibitor to be tested were made up in 1 per cent weight or volume concentrations, depending upon whether the inhibitor was a solid or a liquid, and diluted to the various concentrations with water. It was found that consistent results could be obtained when the inhibiting concentration of each successive dilution was reduced so that each step had one-half the concentration of the preceding one. Steps at smaller intervals did not give easily reproducible results. A few compounds, insoluble in water,

[1] A contribution of the Carver Foundation, Tuskegee Institute.
This investigation was supported in part by a grant from the Parker Pen Company.
[2] In partial fulfillment of the requirements for the Master of Science degree, Tuskegee Institute.

were dissolved in 2–5 ml. of Cellosolve and then diluted with water. An equal amount of Cellosolve was used in the control. Other insoluble compounds were added to a small amount of 2.5 per cent NaOH solution and diluted. These compounds were acidic and the base tended to neutralize them. Their effect on the pH of the medium was minimized still further by the dilutions used. Compounds insoluble in water, Cellosolve, or NaOH were heated in water, the solution filtered, and the filtrate tested for fungicidal properties. When the solubility was recorded in the literature, the literature value was used to calculate the percentage concentration in the medium. In instances where the solubility of a solid compound shown to be a good inhibitor was not available, it was determined experimentally as follows: A known quantity of the solid was stirred with 100 ml. of hot distilled water for 30 min., cooled, and filtered through a previously weighed filter paper which had been dried in a desiccator. The filter paper and residue were dried and weighed, and simple subtraction gave the weight of dissolved material. The concentration in the broth could be calculated from this figure. The solubilities of compounds numbered 4, 28, 38, and 39 were determined in this manner.

Five ml. of each concentration of inhibitor were added to exactly 5 ml. of sterilized broth solution, the tubes inoculated with 0.1 ml. of an *A. niger* suspension, and incubated at 37° C. After 48 hr. the tubes were examined microscopically for mold growth. Controls were run in all cases. The lowest percentage concentration of the inhibiting chemical in the broth solution at which the organism failed to grow was taken as the inhibiting concentration.

RESULTS

Tables 1 through 8 give the effects of 82 readily obtainable compounds on the growth of *A. niger*. The number of the compound is a measure of the relative effectiveness of those tested; the lower the number, the more effective is its inhibiting activity. The term "sat'd" indicates that a saturated solution was added to the broth, while (+) after the percentage concentration indicates that growth was not inhibited at the concentration given. The names of the compounds were selected so as to show their structural relationships, and are not necessarily the ones commonly used.

Cetab, Rodalon, Octimet, and Germ-I-Tol are trade names of quaternary ammonium compounds or mixtures of them. The relationship between chemical constitution and germicidal activity of this class of compounds has been investigated by Rawlins (12) and LoCicero (10) and their co-workers. Table 8 shows that these quaternary ammonium compounds are as effective as any other class tested.

In general, an examination of the tables shows that quaternary ammonium compounds and chloro-alkyl or chloro-nitro substituted phenols will inhibit mold growth at much lower concentrations than the more commonly used chemicals.

EFFECT OF HYDROCARBON SUBSTITUENTS

Tilley and Schaffer (15) showed that the presence of alkyl groups in phenols increased the phenol coefficient, while Klarmann and co-workers (5, 8) extended this generalization to halogenated phenols, resorcinol, and phloroglucinol. The data in table 1 indicate that 2- or 4- alkyl substitution

TABLE 1.—*Effect of hydrocarbon substituent*

No.	Compound	Inhibiting concentration
3.	4-phenylphenol	0.0038
10.	2-cyclohexylphenol	0.0075
12.	4-*tert.*-amylphenol	0.0075
13.	2-phenylphenol, sodium salt (Dowicide A)	0.010
15.	2-isopropylphenol	0.015
16.	mixture of di-isopropylphenols	0.015
23.	4-benzylphenol	0.031
26.	4-*tert.*-butylphenol	0.031
31.	4-methylphenol	0.060
32.	phenol	0.060
53.	3-*tert.*-butylphenol	sat'd
62.	2-(2,3-dihydroindene) phenol	sat'd (+)
8.	2-chloro-4-phenylphenol, sodium salt	0.0075
22.	2-chlorophenol	0.03
37.	2-hydroxy-4-*tert.*-butylphenol	0.063
75.	2-hydroxyphenol	1.0 (+)
28.	thymoquinone	0.047
40.	quinone	0.13

in phenols, quinone, and catechol produces compounds with increased inhibitory power. As indicated by Cavill et al. (2), ring alkyl or aryl groups attached directly to the aromatic nucleus (No. 3, 10, 13) are more effective than benzyl (No. 23). Secondary or tertiary alkyl groups (No. 12, 15, 16, 26) are more effective substituents than is the methyl (No. 31); while the number of alkyl groups attached to the same ring seems not to be a determining factor.

EFFECT OF HALOGEN SUBSTITUENTS

Felton and Brewer (3) have shown that halogen in the aromatic nucleus increases the inhibitory effectiveness of salicylaldehydes, while Suter (14), Cavill (2), Klarmann et al. (7), and others have shown that halogen substitution acts similarly in other compounds. This information is, in general, substantiated in table 2. 2-Chloro or 2-bromo substitution in 4-phenylphenol, however, does not follow the general rule (No. 3, 8, 9, 25). There seems to be no adequate explanation for this. Felton and Brewer (3) found little relationship between inhibitory action and the kind of halogen, while Klarmann and von Wowern (9) found chlorine substitution to be more effective than bromine, and Woodward (16) showed that effective action is increased in the order Cl, Br, I. While the test results on the two compounds containing chlorine or bromine (No. 8, 25) do not give sufficient evidence upon which to make a generalization, it can be noted that the data do not agree with those of Woodward.

In contrast with the work of Felton and Brewer (3) and in agreement with that of Jensen (4), the effect of multiple halogen substitution seems to follow no fixed pattern (No. 17, 18, 19, 22, 24).

TABLE 2.—*Effect of halogen substituent*

No.	Compound	Inhibiting concentration
18.	3-chlorophenol	0.015
19.	4-chlorophenol	0.015
22.	2-chlorophenol	0.030
32.	phenol	0.060
11.	2-chloro-4-nitrophenol	0.0075
20.	4-nitrophenol, sodium salt	0.025
3.	4-phenylphenol	0.0038
8.	2-chloro-4-phenylphenol, sodium salt (Dowicide D)	0.0075
9.	mixture of 2- and 4-chloro-6-phenylphenol, sodium salt (Dowicide 31)	0.0075
25.	2-bromo-4-phenylphenol, sodium salt (Dowicide 5)	0.031
5.	2,2′-methylene bis (4-chlorophenol)	0.0063
7.	2,4,5-trichlorophenol, sodium salt (Dowicide B)	0.0075
17.	pentachlorophenol, sodium salt (Dowicide G)	0.015
24.	2,3,4,6-tetrachlorophenol, sodium salt (Dowicide F)	0.031

Particularly interesting is 2,2′-methylene-bis (4-chlorophenol) which displays excellent inhibitory properties that can probably be attributed to the double 4-chlorophenol grouping.

EFFECT OF NITRO SUBSTITUENTS

Woodward (16) found that the addition of nitro groups to dihydroxy compounds had no effect on the inhibitory properties of these compounds. Cavill et al. (2) showed that nitro in phenol and aniline increased the inhibitory properties, but in benzoic acid the effect was just the opposite. The results in table 3 indicate that, with the exception of 3-nitrobenzoic

TABLE 3.—*Effect of nitro substituent*

No.	Compound	Inhibiting concentration
11.	2-chloro-4-nitrophenol	0.0075
14.	4-nitrobenzoic acid	0.013
20.	4-nitrophenol, sodium salt	0.025
21.	2-nitrophenol	0.025
22.	2-chlorophenol	0.030
27.	2-methyl-3,5-dinitrophenol	0.031
29.	3-nitrophenol	0.050
32.	phenol	0.060
36.	benzoic acid	0.063
45.	3-nitrobenzoic acid	0.17
50.	2-amino-4-nitrophenol	0.25
56.	2-isopropyl-5-methyl-4,6-dinitrophenol	sat'd (+)
64.	2-aminophenol	sat'd (+)

acid (No. 45), a nitro group increases inhibitory effectiveness in phenol, chlorophenol, aminophenol, and benzoic acid (No. 29, 32; 11, 22; 50, 64; 14, 36). The introduction of more than one nitro group probably results in compounds too insoluble to have inhibitory properties (No. 56).

EFFECT OF GROUPS CONTAINING NITROGEN OTHER THAN NITRO

With the exception of nitro, compounds containing nitrogen attached to the ring (Table 4) have weaker inhibiting powers than the parent com-

TABLE 4.—*Effect of groups containing nitrogen (other than nitro)*

No.	Compound	Inhibiting concentration
20.	4-nitrophenol, sodium salt	0.025
32.	phenol	0.060
41.	salicylic acid	0.13
46.	salicylanilide	0.25
50.	2-amino-4-nitrophenol	0.25
60.	benzeneazoresoreinol	sat'd (+)
61.	2-amino-4-sulfophenol	sat'd (+)
64.	2-aminophenol	sat'd (+)
65.	4-aminophenol	sat'd (+)
76.	3-hydroxyphenol	1.0 (+)
77.	3-hydroxyphenol-2-aminohydrochloride	1.0 (+)
78.	3-hydroxy-4-nitrosophenol, sodium salt	1.0 (+)
79.	4-acetamidophenol	1.0 (+)
81.	3-aminophenol	1.0 (+)
82.	2,4-diaminophenoldihydrochloride	1.0 (+)

pound. Thus, the aminophenols (No. 64, 65, 81, 82) and 4-acetamidophenol (No. 79) are weaker inhibitors than is phenol (No. 32); 2-amino-4-phenol (No. 50) is weaker than 4-nitrophenol (No. 21); salicylanilide (No. 46) is weaker than is salicylic acid (No. 41); while benzeneazoresorcinol (No. 60), 3-hydroxy-4-nitrosophenol (No. 78), 2-amino-4-sulfophenol (No. 61), and 3-hydroxyphenol-2-aminohydrochloride (No. 77) show no inhibiting properties at 1 per cent or saturated concentrations.

INFLUENCE OF CARBOXY AND SULFONIC ACID GROUP

With the possible exception of benzoic acid (No. 36), aromatic compounds having a carboxy or sulfonic acid group have weak inhibiting properties or none. Table 5 shows that these groups, when introduced into

TABLE 5.—*Effect of carboxy and sulfonic acid groups*

No.	Compound	Inhibiting concentration
32.	phenol	0.060
36.	benzoic acid	0.063
41.	salicylic acid	0.13
44.	benzaldehyde	0.15
45.	3-nitrobenzoic acid	0.17
55.	benzyl salicylate	sat'd (+)
61.	2-amino-4-sulfophenol	sat'd (+)
66.	2-hydroxy-3-methyl benzoic acid	1.0 (+)
67.	3,4,5-trihydroxy benzoic acid	1.0 (+)
68.	2,4-dihydroxy benzoic acid	1.0 (+)
69.	sodium salicylate	1.0 (+)
70.	5-sodium sulfosalicylic acid	1.0 (+)
73.	2-sodium sulfobenzaldehyde	1.0 (+)

compounds having other groups, seem to have an adverse effect on inhibiting power (No. 41, 70; 44, 73; 32, 41).

INFLUENCE OF HYDROXY GROUPS

As indicated by Suter (14), the presence of one hydroxy group in an aromatic ring leads to inhibitory powers, but when a second is added, the inhibitory power is lessened. The following groups of compounds in table 6 show this effect: No. 26, 37; 41, 68; 32, 76, 75; 31, 74. Likewise,

TABLE 6.—*Effect of hydroxy groups*

No.	Compound	Inhibiting concentration
26.	4-*tert.*-butyl phenol	0.031
31.	4-methylphenol	0.060
32.	phenol	0.060
33.	2,4-dihydroxy benzaldehyde	0.063
36.	benzoic acid	0.063
37.	2-hydroxy-4-*tert.*-butylphenol	0.063
41.	salicylic acid	0.13
67.	3,4,5-trihydroxy benzoic acid	1.0 (+)
68.	2,4-dihydroxy benzoic acid	1.0 (+)
74.	3-hydroxy-5-methylphenol	1.0 (+)
75.	2-hydroxyphenol	1.0 (+)
76.	3-hydroxyphenol	1.0 (+)

gallic acid, with three hydroxy groups (No. 67), is less toxic to *A. niger* than is benzoic acid (No. 36).

INFLUENCE OF POSITION

In general, the position of a group influencing inhibitory properties is important. As observed by Browning et al. (1), Klarmann (7), Suter (14), Morgan (11), and Schaffer and Tilley (13), *p*-substitution is more effective in increasing inhibitory power than is *o*- or *m*- substitution. This observation is somewhat substantiated by the nitrobenzoic acids and the nitro-, chloro-, and phenyl-substituted phenols (Table 7). Conclusions as

TABLE 7.—*Effect of position*

Compounds	Inhibiting concentration		
	ortho	*meta*	*para*
Nitrobenzoic acids	.....	0.17	0.013
Aminophenols	sat'd (+)	1.0 (+)	sat'd (+)
Nitrophenols	0.025	0.050	0.025
Chlorophenols	0.030	0.015	0.015
Phenylphenols	0.010	.....	0.0038

to the relative effectiveness of the *m*-position cannot be drawn from the existing data.

In the present work some compounds were tested which structurally did not fall into the groups already discussed. The data on these compounds

are not at present particularly useful in correlating structural relationships and inhibitory effectiveness, but their listing in table 8 allows a comparison of their inhibiting concentrations with those of the compounds in the other tables.

The suppliers and the numbers of the compounds supplied are as follows: Eastman Kodak Company, 3, 10, 11, 12, 14, 18–23, 26–29, 33, 37–39, 43, 45, 50, 53–56, 58–63, 66, 67, 70, 73, 74, 77–80, 82; J. T. Baker Chemical Company, 31, 32, 34, 36, 40, 41, 47, 75, 76; Dow Chemical Corporation, 7–9, 13, 17, 24; Fisher Scientific Company, 42, 44, 72; Koppers Company, Inc.,

TABLE 8.—*Inhibitory concentration of miscellaneous compounds*

No.	Compound	Inhibiting concentration	
1.	Cetab (hexadecyltrimethyl ammonium bromide)	0.0038	
2.	Rodalon (90 per cent alkyldimethylbenzyl ammonium chloride)	0.0038	
4.	3-isopropyl-4-chloro-6-methylphenol	0.0049	
6.	Octimet (85 per cent octadecyldimethylethyl ammonium bromide, 15 per cent octadecenyldimethylethyl ammonium bromide)	0.0075	
30.	Propyl Parasept (propyl-4-hydroxy benzoate)	0.050	
34.	furfural	0.063	
35.	acetylsalicylic acid	0.063	
38.	2-hydroxy-5-chlorobenzaldehyde	0.066	
39.	*alpha-n*-amyl cinnamaldehyde	0.10	
42.	formaldehyde	0.13	
43.	2-methoxy-4-methylphenol	0.13	
47.	benzyl alcohol	0.25	
48.	Methyl Parasept (methyl-4-hydroxy benzoate)	0.25	
49.	Germ-I-Tol (dimethylbenzylalkyl ammonium chloride)	0.25	
51.	ethylene thiourea	0.50	
52.	2-isopropyl-4-chloro-5-methylphenol	sat'd	
54.	resorcinol dibenzoate	sat'd	(+)
57.	benzoin	sat'd	(+)
58.	dihydroxy anthraquinones (65 per cent 1,5; 35 per cent 1,8)	sat'd	(+)
59.	phenyl isothiocyanate	sat'd	(+)
63.	*p*-benzalaminophenol	sat'd	(+)
71.	phenyl urea	1.0	(+)
72.	*p*-aminobenzene sulfonamide	1.0	(+)
80.	4-acetamidoacetyl phenol	1.0	(+)

15, 16, 68; Rhodes Chemical Corporation, 1, 2, 7; Tenn. Eastman Corporation, 64, 65, 81; Heyden Company, 30, 48; Orbis Products Corporation, 3, 52; Fine Organics, Incorporated, 49; Rohm and Haas, 51; Sindar Corporation, 5. The remaining compounds were synthesized in this laboratory by the usual methods. We acknowledge with gratitude the research samples furnished by Dow Chemical Company; Koppers Company, Inc.; Rhodes Chemical Corporation; Fine Organics, Incorporated; Heyden Company; Orbis Products Corporation; Rohm and Haas, and the Sindar Corporation.

SUMMARY

Of 82 compounds tested for inhibitory action against *A. niger*, the most effective are some of the quaternary ammonium compounds and the alkyl-

and chloro- substituted phenols. Substituent groups listed in order of their ability to increase inhibitory effectiveness are: ring alkyl, aryl, secondary- or tertiary- alkyl, halogen, and nitro. Groups containing nitrogen other than nitro, the carboxy, sulfonic acid, and multiple hydroxy groups, do not enhance inhibitory properties. *Para* substitution is more effective than is *ortho* or *meta*.

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HERITABILITY OF NUMBER OF PRIMARY SPORIDIA PRODUCED BY SPORES OF TILLETIA CARIES AND T. FOETIDA[1]

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Physiologic races of *Tilletia caries* (DC.) Tul. and *T. foetida* (Wallr.) Liro differ in many respects (1, 2), including the number of primary sporidia produced by germinating chlamydospores. Hanna (1) concluded that the number of sporidia produced tended to be influenced by environmental factors such as temperature, but that the capacity of the spores to produce large or small numbers of sporidia probably is a heritable character. The heritability of other race characters has been demonstrated (4).

In recent studies on the bunt fungi, the writers also observed consistently distinct differences in the number of primary sporidia borne by the promycelia of different races of both species. Studies concerning the heritability of that character are reported here.

MATERIALS AND METHODS

Two races of *Tilletia caries* (T-9 and C.T.13[3]) and two races of *T. foetida* (L-1 and L-9) (5) were used in these experiments. Chlamydospores of each race were collected from winter wheat grown in the smut nursery at Pullman, Washington. The primary sporidia produced on individual promycelia from germinating chlamydospores were counted with the aid of a micromanipulator. The number of spores on which counts were made ranged from 24 in the L-1 and L-9 races to 208 in the T-9 and C.T.13 races. The relatively small number counted for the L-1 and L-9 races was sufficient to establish an approximately normal frequency distribution. Much larger numbers appear to be unnecessary.

Monosporidial lines (cultures) of each race were obtained by isolating complete sets of primary sporidia from the promycelia of germinating chlamydospores and transferring each sporidium to a separate tube of potato-dextrose agar. Complete sets of cultures were not always obtained since some of the isolated sporidia failed to grow. For example, T-9 was represented by six cultures from a complete set of seven sporidia produced by spore 148, and C.T.13 was represented by a complete set of eight cultures from spore 153. Only three cultures of L-1 survived from a set of eight sporidia produced by spore 180. Nine cultures were obtained from a set of 17 sporidia produced by spore 181 of L-9. Inoculations were made

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[2] Pathologists, Bureau of Plant Industry, Soils, and Agricultural Engineering.

[3] Collection No. 13 of *Tilletia caries* (5).

with paired monosporidial lines by the method previously described (3), using the susceptible variety Hindi.

The chlamydospores of T-9, C.T.13, and the hybrids between them were germinated on 2 per cent water agar at approximately 18° C. The spores of L-1, L-9, and their hybrids were germinated on 4 per cent water agar at two temperatures, 8° C. and 12°–15° C.

EXPERIMENTAL RESULTS

Parent races. The number of primary sporidia produced by the four races are in table 1. The mean number of sporidia produced at the higher

TABLE 1.—*Number of sporidia produced by two races each of Tilletia caries and T. foetida*

Race	Temperature (° C.)	Number of spores	Number of primary sporidia produced		
			Range	Mode	Mean
T-9	18	201	7–16	12	11.3
C.T.13	18	200	5–11	7	7.4
L-1	8	46	3–9	5, 6	5.4
	12–15	70	3–11	6	6.7
L-9	8	43	7–16	11	10.8
	12–15	96	6–23	12	12.9

temperatures ranged from 6.7 to 12.9 among the four races. The number of sporidia produced by the L-1 and L-9 races was slightly larger at the higher temperature range, but the relative difference between the two races remained approximately the same.

The wide range in number of sporidia produced by different chlamydospores of the same race (Table 1) emphasized the importance of determining the genotype of each monosporidial line entering into a cross. Attempts were made to produce pure line chlamydospores of each parent race by inbreeding, but inbred lines were obtained from only one race (T-9). Data on the number of sporidia produced by the six inbred lines of this race are in table 2. The mean number of sporidia ranged from 8.9 to 14.1.

TABLE 2.—*Pedigree and number of sporidia produced by six inbred lines of T-9 derived from chlamydospore 148*

Monosporidial combination	Inbred line number	Number of spores	Number of primary sporidia produced		
			Range	Mode	Mean
3 × 4	4	101	7–15	8	8.9
1 × 6	2	100	7–15	8	9.8
5 × 6	6	102	6–19	10	10.1
1 × 3	1	100	6–18	9	11.5
2 × 3	3	101	7–19	12	11.5
3 × 5	5	100	7–25	14	14.1

These figures suggest two possible factors controlling the number of sporidia produced by this chlamydospore. Combinations in which monosporidial

lines 4 and 6 were involved produced fewer than did those involving the other four lines.

From the data in table 2 it appears that inbred lines 1 and 3 are more representative of T-9 (Table 1) than the others. This is an important consideration in studying hybrids with T-9, since monosporidial lines 1, 2, and 3 appear to carry factors for large numbers of sporidia. Thus, hybrids with any one of these three monosporidial lines as one parent should reflect the high sporidial production of the T-9 parent in the segregating generation. On the other hand, hybrids with monosporidial lines 4 and 6 might not reflect the T-9 parent in this respect.

Hybrids. Ten hybrids were obtained from inoculations with all possible combinations between six monosporidial lines of T-9 (spore 148) and eight monosporidial lines of C.T.13 (complete set from spore 153). Monosporidial lines 3 or 6 of T-9 entered into all of these hybrids. The mean number of sporidia produced by the 10 hybrids ranged from 7.8 to 9.3 (Table 3). In this respect, therefore, these hybrids resemble the C.T.13 parent more than the T-9 parent (Tables 1 and 3).

TABLE 3.—*Number of primary sporidia produced by ten hybrids between races T-9 and C.T.13*

Spore and monosporidial combination	Hybrid number	Number of spores	Number of primary sporidia produced		
			Range	Mode	Mean
148 × 153					
3 × 2[a]	110	204	5–11	8	7.8
3 × 6	113	202	5–12	8	7.9
3 × 1	109	200	5–15	8	8.3
3 × 5	112	203	5–13	8	8.5
3 × 7	114	207	5–15	8	8.6
3 × 3	111	208	5–16	9	8.6
6 × 2	115	201	5–16	9	8.5
6 × 3	116	200	5–16	7	8.7
6 × 6	118	72	5–17	8	9.2
6 × 5	117	206	5–15	10	9.3

[a] Monosporidial line 3 from spore 148 of T-9 crossed with monosporidial line 2 from spore 153 of C.T.13.

Dominance of factors for small numbers of sporidia in the C.T.13 parent is indicated in the six hybrids involving monosporidial line 3 of the T-9 parent. If this line carried a factor for large numbers of sporidia (Table 3), it apparently was recessive to the factor for small numbers of sporidia found in C.T.13. Monosporidial line 6 of T-9 apparently produced small numbers of sporidia also, as might be anticipated.

Seven hybrids between races L-1 and L-9 were obtained. The pedigree and number of sporidia produced by these hybrids are in table 4. The mean number of sporidia ranged from 11.4 to 19.8. Only one of the seven hybrids (No. 1715) had fewer sporidia than did the L-9 parent. The mean number of sporidia produced by the parent races was 6.7 and 12.9 for L-1

TABLE 4.—*Number of primary sporidia produced by ten hybrids between races L-1 and L-9, at 12°–15° C.*

Spore and monosporidial combination	Hybrid number	Number of spores	Number of primary sporidia produced		
			Range	Mode	Mean
180 × 181					
3 × 6[a]	1715	26	8–18	11	11.4
2 × 8	1708	44	7–23	12, 13	13.5
2 × 1	1701	30	10–21	13, 14, 15	14.4
3 × 1	1710	24	9–21	12, 13	14.6
1 × 6	1697	25	11–23	14	15.4
1 × 9	1700	30	11–20	14, 15	15.5
3 × 3	1712	64	13–30	20	19.8

[a] Monosporidial line 3 from spore 180 of L-1 crossed with monosporidial line 6 from spore 181 of L-9.

and L-9, respectively (Table 1). In this cross, either the factor for large number of sporidia was dominant, or complementary genes were responsible for the large number of sporidia, particularly in hybrid 1712.

Segregates. Complete sets of primary sporidia were isolated from hybrid chlamydospores to produce a segregating generation. Inoculations were made with all possible combinations of monosporidial lines from individual hybrid spores. A complete set of eight monosporidial lines from spore 158 of hybrid 109 produced 12 segregates out of 28 possible combinations. The mean number of sporidia produced by these 12 segregates ranged from 10.4 to 22.8 (Table 5). This indicates probable segregation

TABLE 5.—*Number of sporidia produced by 12 segregates from chlamydospore 158 of hybrid 109*

Sporidial combination	Segregate number	Number of spores	Number of primary sporidia produced		
			Range	Mode	Mean
5 × 8	104	52	6–19	7	10.4
3 × 5	99	53	7–18	10	10.8
5 × 7	103	50	7–16	10, 11	11.4
4 × 5	101	50	8–18	11	11.9
6 × 8	106	52	8–16	12	11.9
1 × 6	96	50	7–17	14	12.1
4 × 6	102	50	7–18	10, 13	12.4
3 × 6	100	53	9–17	16	12.9
6 × 7	105	51	7–20	15	13.5
1 × 5	95	52	9–21	14	14.1
2 × 6	98	50	10–24	20	18.9
2 × 5	97	56	11–30	17, 23	22.8

and recombination of factors controlling the number of sporidia produced. None of the segregates, however, produced as few sporidia as did the C.T.13 parent (7.4). Two segregates (99, 104) were intermediate between the parent races, and six were about the same as the T-9 parent (11.3). Strong transgressive inheritance is evident in segregates 97 and 98, which produced means of 22.8 and 18.9 sporidia, respectively. Both of these segregates had monosporidial line 2 in common, which apparently carried a fac-

tor that was complementary to the factor in monosporidial lines 5 and 6 (Table 5).

Twenty-five segregates were obtained from chlamydospore 166 of hybrid 112 (Table 3). These were produced by the compatible matings among 45 possible combinations of the complete set of 10 monosporidial lines. The mean numbers of sporidia produced by these segregates ranged from 9.2 to 13.4 (Table 6). Most of these 25 segregates resembled the T-9 parent in

TABLE 6.—*The number of sporidia produced by 25 segregates from chlamydospore 166 of hybrid 112*

Sporidial combination	Segregate number	Number of spores	Number of primary sporidia produced		
			Range	Mode	Mean
5×10	219	52	5–14	9	9.2
7×9	221	51	5–17	8	9.4
3×8	212	51	8–15	8	10.5
2×10	208	50	6–15	12	10.6
9×10	223	50	6–17	13	10.7
8×9	222	50	5–16	14	10.8
1×4	199	50	7–16	9	10.9
1×7	201	50	6–17	9	10.9
2×6	205	51	7–16	10	10.9
2×8	207	50	6–16	11	10.9
6×9	220	50	6–19	10, 13	11.2
3×7	211	53	7–16	11	11.3
4×9	215	50	7–17	8, 14	11.2
1×10	203	55	7–16	10	11.4
5×8	218	51	7–16	12	11.4
1×8	202	52	6–17	13	11.6
1×6	200	52	5–17	9	11.7
5×7	217	50	7–17	14	11.7
3×4	209	50	7–17	13	11.8
2×4	204	50	8–17	12	12.2
2×7	206	50	7–19	13, 14	12.3
3×6	210	50	9–16	12	12.5
3×10	213	49	7–17	10	12.5
4×5	214	50	7–18	13	12.7
5×6	216	52	8–22	14	13.4

numbers of sporidia produced. Only two (219, 221) had less than 10 primary sporidia and none had as few as the C.T.13 parent (7.4) or hybrid 112 (8.5). Thus, there is no good evidence of segregation and recombination of factors resulting in a small number of sporidia characteristic of the C.T.13 parent. Also, the indication of dominance for small number of sporidia in C.T.13, mentioned previously, is not confirmed by these results.

Similar results were obtained among 10 segregates from spore 172 of the same hybrid (112). These segregates were produced by the compatible combinations of 21 possible combinations among a complete set of seven monosporidial lines. The mean number of sporidia produced by these 10 segregates ranged from 10.7 to 13.8, thus reflecting the T-9 but not the C.T.13 parent.

Only one segregate was obtained from the hybrids between L-1 and L-9. This was out of hybrid 1700 (Table 4). The mean number of sporidia

produced by 44 spores of this segregate was 13.4, which resembles the L-9 parent (Table 1).

SUMMARY AND CONCLUSIONS

There were differences in number of primary sporidia produced by two physiologic races each of *Tilletia caries* and *T. foetida;* the mean numbers ranged from 6.7 to 12.9 among the four races. The evidence presented indicates that number of sporidia produced by different races is heritable. However, the nature of this inheritance has not been established.

Evidence regarding dominance and the number of genetic factors involved is conflicting. In one experiment there were indications of single-factor differences for number of primary sporidia produced, but this was not confirmed by later results. Likewise, dominance for small numbers was indicated in some combinations but not in others.

A genetic interpretation of the F_2 population is difficult, since no specific pattern of segregation and recombination of factors was indicated. Most of the segregates from hybrids between T-9 and C.T.13 produced large numbers of sporidia as did the T-9 parent, a few produced intermediate numbers, and at least two produced larger numbers of sporidia than the T-9 parent. These results are at variance with the evidence of dominance for small number of sporidia. The production of larger numbers of sporidia than either parent by some of the segregates indicates the action of complementary factors.

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A CANKER DISEASE OF THE COFFEE TREE IN COLOMBIA AND VENEZUELA

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A widespread disease of the coffee tree (*Coffea arabica* L.), known by growers as llaga macana, was brought to the writer's attention during his stay at the National Research Center for Coffee at Chinchina, Colombia, from 1947 to 1949. The disease appeared to resemble the "kanker" described in Java by Zimmermann (15) in 1900 and attributed by him to the fungus *Rostrella coffeae* Zimm. Although Obregon-Botero (11) assigned the cause of the disease to this organism in 1936, no evidence was given that the fungus was reisolated from inoculations made by him. Furthermore, doubt was cast on previous work upon the nature of the disease in 1945 by Mejia-Franco (9, p. 126), who claimed *Fusarium* sp. to be the cause.

Because of the disagreement over the etiology of the disease, and its economic importance, an investigation was undertaken to determine the cause of the canker as it occurs in Colombia. Later, the disease was also found in Venezuela.

SYMPTOMATOLOGY

The first symptom of the disease visible to a person standing at the border of an affected plantation is the faded appearance of the leaves. The foliage turns progressively from yellowish green to yellow. The bark of a diseased tree may appear normal, but brown to black irregularly shaped lesions or cankers of various sizes are revealed when the outer surface of the trunk or main branches is scraped away. Although lesions are most common at pruning wounds or injuries, they may be found at any height and location on the tree. Sometimes several lesions are found on the same tree. However, the canker does not usually appear until trees are 8–10 years of age, and seldom on limbs until bark has been formed for 3 or more years.

The disease progresses slowly but moves more rapidly downward toward the root than upward. Penetration of the wood is slight and the wood is rarely stained at a depth exceeding 2 cm. With the gradual increase in size of the lesions and subsequent dying of the affected tissues, complete girdling of the limb or trunk finally ensues. Usually, however, a new shoot, some distance below the lesion, has begun to grow vigorously before girdling has been completed.

[1] Formerly Head of the Department of Plant Pathology, National Research Center for Coffee, Chinchina, Caldas, Colombia. The author wishes to express his thanks to Dr. H. A. Dade for his helpful advice and the remittance of cultures of Ceratostomella; to Dr. W. C. Snyder for his identification of the Fusarium isolated and for assistance in the preparation of the English manuscript; and to Dr. Gonzalo Plaza and Dr. Nelson Delgado for their assistance in the pathogenicity experiments.

Diseased trees may continue to bear some fruit, but yields decline and such trees become unprofitable.

ETIOLOGY

Canker material for these studies was obtained from the plantations of Caldas and Cundinamarca. More than 100 cankers were cultured during the course of the work.

Tissue platings made from the advancing margin of lesions, on potato-dextrose or cornmeal agar plates and incubated at room temperature, almost always yielded a slow-growing fungus resembling *Ceratostomella fimbriata* (Ell. & Halst.) Elliott. Occasionally colonies of *Fusarium solani* (Mart.) App. & Wr. emend. Snyd. & Hans. were obtained.[2]

The *Ceratostomella* grew readily at pH 6.00 on potato-dextrose and malt-extract agars, and at pH 5.6 on cornmeal agar. The three typical spore forms described for *Ceratostomella fimbriata* (6) were obtained on these media, with characteristics as follows.

The hyaline, cylindrical, continuous conidia varied extremely in size, measuring 9.5 μ–49.2 μ long in an 8-day-old culture on potato-dextrose agar. These were capable of immediate germination after separation from the chains in which they were produced. Zimmermann (15) reported as many as 46 conidia in a chain.

The oval, olive-brown, thick-walled conidia or chlamydospores ranged from 10.5 $\mu \times$ 18.8 μ to 7.7 $\mu \times$ 10.3 μ, on cornmeal agar after 3 months. Their appearance followed by some time the formation of the hyaline conidia, and they were few in number as in the case of the *Ceratostomella fimbriata* isolated by Davidson (4) from hardwood lumber. Histological studies of diseased tissues showed, however, the production of these dark, oval spores within cells of the host by the intracellular mycelium (Fig. 2, D).

Hyaline ascospores were produced in great numbers in black, membranous perithecia and resembled the hat-shaped ascospores seen in some species of the genus *Hansenula* and in the family Endomycetaceae. The ascospores measured 4.7 μ–7.0 $\mu \times$ 3.2 μ–4.8 μ, on cornmeal agar cultures after 8 days. At maturity these spores were extruded in long gelatinous spore horns or coils from the perithecial cavity. The perithecia are flask shaped (Fig. 2, A, B), range from 103 μ to 150 μ in diameter, and possess long cylindrical beaks which may reach a length of 1500 μ and which are fimbriate at the apex. Although abundant in culture, no perithecia were found on infected tissues in the field.

Asci were not observed in any of the fresh preparations made from perithecia, no doubt because of the early disintegration of the ascus, as shown by Elliott (6). This deliquescence of the ascus is a characteristic of many genera of Ascomycetes but particularly of those in the order *Sphaeriales*. Mittmann (10) and Andrus and Harter (1, 2) have shown that deliquescence is not necessarily a result of wall dissolution, since the

[2] Identified by Dr. W. C. Snyder.

ascus of *C. fimbriata* possesses no wall from the time of its origin, but represents a disorganization of the peripheral region of a naked or plasmodial mass that has already reached a state of senescence. The mature ascus,

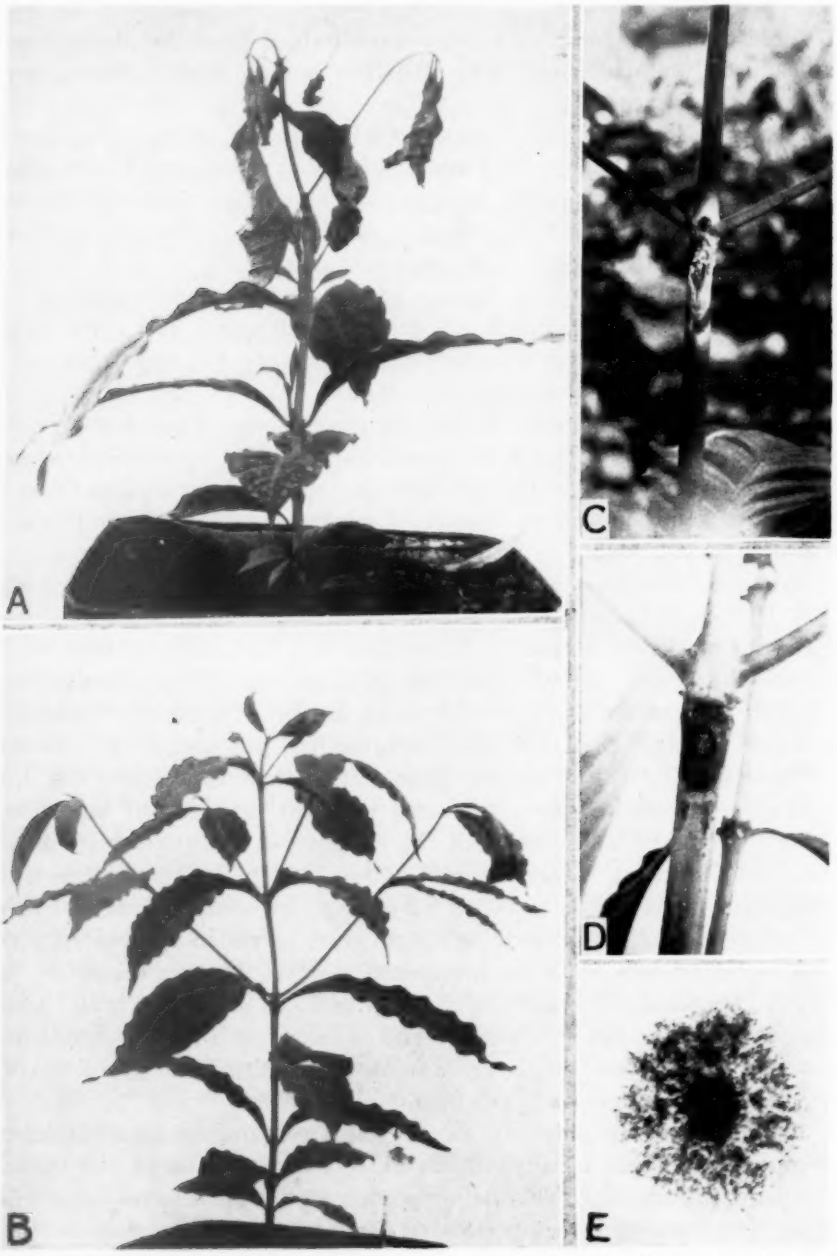

FIG. 1. A. Young coffee tree 37 days after inoculation with *Ceratostomella* isolate from coffee. B. Control tree of the same age. C. Trunk with canker, 50 days after inoculation. D. Another trunk, 20 days after inoculation. E. Culture of *Ceratostomella* from coffee, 11 days old.

then, consists of a compact group of spores to which are attached remnants of inert cytoplasm.

PATHOGENICITY TESTS

Since *Fusarium* sp. has been reported (9) as the causal agent of coffee canker, several inoculation experiments were made with the Colombia isolate of *F. solani* recovered from diseased trees.

Mycelium and spores from 13-day-old cultures on potato-dextrose agar were used to inoculate the main trunks, at various heights, of coffee trees of different ages after the method described for citrus trees by Klotz and Fawcett (8). Sterile medium was placed in an equal number of noninoculated trees to serve as controls. In 1947, 15 trees were inoculated with the *Fusarium* on November 3, 20 on December 19, and another 20 on January

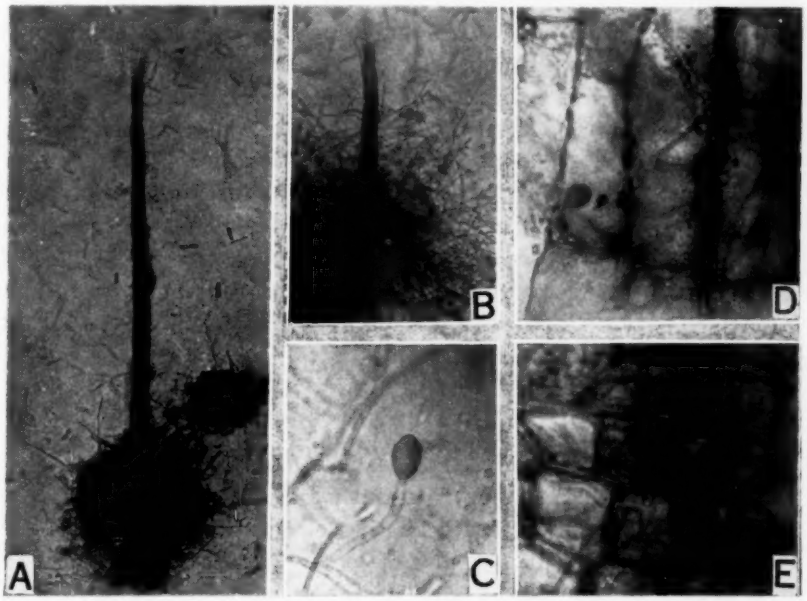

FIG. 2. Coffee canker fungus. A and B. Mature perithecia. C. Oval olive-brown conidium. D and E. Oval conidium and mycelium in the cells.

5, 1948. All inoculations gave negative results. It is concluded that the *F. solani* used in these tests is a secondary fungus which invades diseased areas and which may even coexist with the mycelium of the pathogen near the margin of the cankers, but is itself not the pathogen.

Inoculations with the *Ceratostomella* isolate were made by inserting mycelium with perithecia from 12-day-old cornmeal agar plates into vertical wounds made with a flamed scalpel and then covering the wounds with adhesive tape. Ten coffee trees, 10 months old, were inoculated in this manner on February 10, 1948. Seven days later symptoms began to appear in two trees, and two days later all trees were infected. The control trees remained healthy. The first visible symptom was a small spot of blackened tissue near the point of inoculation. Fifty days later the lesions

were larger (Fig. 1, C) but after 5 months the lesions had ceased to enlarge further in eight of the trees as a result of cicatricial tissue similar to that observed in young branches by Delacroix (5, p. 350).

A second group of 20 3-year-old trees was inoculated on April 12 and another group of 25 trees on April 23, again using the method of Klotz and Fawcett. Twenty days later 100 per cent of the trees were infected. Reisolations made from the margins of the lesions consistently yielded a *Ceratostomella* identical with that used for inoculation.

The results from these inoculation experiments left no doubt that the cause of the coffee-tree canker, or llaga macana, is a species of *Ceratostomella*.

Since the *Ceratostomella* from coffee so closely resembled *C. fimbriata*, cross-inoculation experiments were made, employing pathogens of both the coffee tree and the sweet potato. Twenty sweet-potato roots, obtained from an undetermined variety found growing out of cultivation in the region, were inoculated by dipping in a spore suspension of conidia, ascospores, and mycelium removed from a culture of the coffee isolate and incubated at room temperature. At the same time, 20 coffee trees of different ages were inoculated with an isolate of the sweet-potato fungus, *C. fimbriata*, sent to the writer by W. J. Martin of Louisiana State University. None of these cross inoculations produced infection.

The related species of fungi, *Thielaviopsis paradoxa* and *Ceratostomella pilifera*, were also tested for pathogenicity on coffee. Of 25 trees inoculated with each fungus none became infected.

TAXONOMY

It is believed from a study of the canker disease of coffee in Colombia that it is the same as that known in Java under the name of "kanker." Furthermore, Zimmermann's pathogen described under the name *Rostrella coffeae* Zimm. is in morphological agreement with the species of *Ceratostomella* proven to cause the disease in Colombia.

The genus *Rostrella* was set up by Zimmermann (15) in 1900. A description of the genus, and of the species *R. coffeae*, may be found in Saccardo (12, p. 609). This description is believed to be in essential agreement with that of the coffee pathogen given here.

Since Zimmermann's *Rostrella* from coffee appears to be the same fungus as the writer's *Ceratostomella* from coffee, and since the latter genus is the older of the two, having been described in 1878 by Saccardo, the genus name *Ceratostomella* has preference over *Rostrella* in this instance. Furthermore, since the coffee pathogen is similar in morphology to *C. fimbriata*, even though preliminary cross-inoculation tests indicate that it is biologically distinct from the black-rot fungus of sweet potato, it is proposed that the fungus causing the canker of the coffee tree (*Coffea arabica*) be assigned to the species *Ceratostomella fimbriata*.

If, however, we were to follow Davidson's (4) taxonomy of this group of fungi, the name proposed would be *Endoconidiophora fimbriata* (Ell. & Halst.) Davidson.

A herbarium specimen and culture of the Colombia fungus were sent to Dr. H. A. Dade for deposit in the herbarium of the Commonwealth Mycological Institute, Kew, England. In a personal communication, Dr. Dade confirmed the writer's diagnosis and opinion that *Rostrella coffeae* Zimm. is identical with and a synonym of *Ceratostomella fimbriata*.

In addition to causing a canker of the coffee tree, the morphological species *Ceratostomella fimbriata* produces the black rot of sweet potato (7), moldy rot of tapping panels of Hevea rubber trees (13, pp. 208–229), a disease of Crotalaria (3), a stain of logs and lumber in southern United States (4), and a canker stain of planetrees (14).

SUMMARY

A canker disease of the coffee tree in Colombia and Venezuela, known locally as llaga macana, causes widespread economic loss in production. It is believed to be the same disease as "kanker," reported on coffee trees in Java.

Cankers of the trunk and branches may girdle the affected parts. They usually occur around pruning wounds and injuries on older wood.

A species of *Ceratostomella* was consistently isolated from the cankers and proven by inoculation and reisolation to be the pathogen. A species of *Fusarium* occasionally recovered from cankers was not pathogenic in inoculation tests.

Taxonomic studies show that the *Ceratostomella* is morphologically similar to *C. fimbriata* and it is proposed, therefore, that the coffee pathogen be assigned to this species. *Rostrella coffeae* then becomes a synonym of *C. fimbriata*.

Attempts to induce cankers in the coffee tree with the strain of *C. fimbriata* from sweet potato, and with the related species *Thielaviopsis paradoxa* and *Ceratostomella pilifera*, were unsuccessful.

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PHYTOPATHOLOGICAL NOTES

Suggestions for Transferring Virus from Local Lesions to Test Plants.[1] FRANK P. MCWHORTER.[2] When highly infectious viruses are being studied, transfers from local lesions are often made by teasing the lesion with a needle and then scratching a leaf of the test plant with the same needle. When larger amounts of virus are required, the lesion is cut out, ground, and inoculated upon the test plant. The following procedure has proved effective for viruses that are difficult to transfer.

The ends of small glass or metal tubes are ground until the edges are sharp. (Very small tubes can be made conveniently from the pipettes of dropper bottles.) Rubber nipples are mounted on the tops of the tubes. When a blotter or cardboard is placed under the leaf and the prepared tube is rotated on the lesion, the desired tissue can be quickly and cleanly separated from the leaf. The rubber nipple is then used to blow the tissue onto a surface for grinding.

From ground-glass plates, sold for photographic purposes, squares 1 to 2 in. on a side are cut. A droplet of a suitable diluent is placed on a square with a small glass rod, and the lesion tissue is blown onto the droplet. The tissue is then emulsified in the droplet with a ground-glass spatula prepared according to the original method of Samuel.[3] Spatulas of small size should be provided to accommodate very small pieces of tissue. This rubbing of the tissue on the ground-glass surface with a ground-glass spatula prepares a perfect emulsion almost instantly. The same spatula is then used to make precise transfers of the inoculum to the test plants. It is easy to begin with a local lesion and finish an inoculation in 15 sec. Thus, transfers can be effected without recourse to antioxidants in the diluent.

This technique, when larger spatulas are used, enables one to emulsify quickly portions of very refractory leaves, such as old zinnia leaves. The method is so rapid that it is especially useful in effecting difficult transfers, such as one encounters in transmitting the easily inactivated spotted-wilt virus between some of its widely divergent hosts.—Oregon Agricultural Experiment Station, Corvallis, Oregon.

Maintaining Virulence in Isolates of Pseudomonas solanacearum. ARTHUR KELMAN AND J. H. JENSEN. The bacterial pathogen, *Pseudomonas solanacearum* E. F. Smith, causal agent of southern bacterial wilt, undergoes a relatively rapid loss of pathogenicity in culture. Attenuation of isolates after successive transfers for a number of weeks on standard

[1] Published as Technical Paper No. 622, with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution of the Department of Botany and Plant Pathology.

[2] Plant Pathologist, Oregon Agricultural Experiment Station, and Agent, U. S. Department of Agriculture.

[3] Samuel, Geoffrey. Some experiments on inoculating methods with plant viruses, and on local lesions. Ann. Appl. Biol. 18: 494–507. 1931.

media has been observed by Smith[1] and many other workers. In connection with an expanding breeding program, greenhouse inoculations were considered necessary to expedite evaluation of resistance to southern bacterial wilt in tomato and Irish potato selections. A simple method of maintaining virulence of selected isolates for periods longer than several months was needed for these inoculations.

One effective means of maintaining viability of bacteria consists of covering bacterial cultures on solid media with a layer of sterile mineral oil.[2] The successful application of this technique for retention of virulence by a bacterial plant pathogen was reported by Sherf,[3] who found that cultures of *Corynebacterium sepedonicum* (Spiek. & Kott.) Skap. & Burk. retained viability and pathogenicity for 18 months under the oil. Jensen and Livingston[4] maintained strains of the bean halo-blight organism, *Pseudomonas phaseolicola* (Burk.) Dow., under mineral oil for 4 years with only minor changes in pathogenicity or growth characteristics. In early studies with *P. solanacearum*, E. F. Smith used mineral oil to demonstrate oxygen requirements of the pathogen and noted that a layer of mineral oil which excluded oxygen prevented growth in broth cultures. Experiments to determine whether the mineral oil technique could be utilized to maintain virulence in isolates of *P. solanacearum* are reported herein.

Stock cultures were made in 1945 from isolates obtained from diseased potato, tobacco, and peanut plants. Selected virulent isolates were streaked on potato-dextrose agar slants and after 48 hr. sterile mineral oil (Squibb, medicinal grade) was added to each tube to a level of about 1 cm. above the upper edge of the agar slant. Cultures were then stored at about 25° C. Subcultures for inoculations were made by removing a loop of bacteria from the surface of the slant beneath the oil and transferring to fresh potato-dextrose agar in slants or Petri plates. In order to free each subculture from a residue of oil, a second transfer was usually made. Pathogenicity tests were conducted annually, using Marglobe tomato seedlings with five to seven expanded leaves. Plants were inoculated by forcing a needle into the stem through a drop of bacterial slime scraped from the surface of a 3- to 5-day-old culture. This stem puncture was usually made at the third node below the stem tip. Eight to ten seedlings were inoculated for each isolate. Disease readings were made at 7-day intervals by grading plants individually as to symptoms, using a unit scale from 0 (no wilting symptoms) to 5 (dead). Readings were converted to disease indices by a method similar to that used by Kendrick and Walker[5]. Results obtained with four isolates are summarized in table 1.

[1] Smith, E. F. Bacteria in relation to plant diseases. Vol. 3. 309 pp. Carnegie Institute. 1914.

[2] Morton, H. E., and E. J. Pulaski. The preservation of bacterial cultures. I. Jour. Bact. 35: 163–183. 1938.

[3] Sherf, A. F. A method for maintaining *Phytomonas sepedonica* in culture for long periods without transfer. Phytopath. 33: 330–332. 1943.

[4] Jensen, J. H., and J. E. Livingston. Variation in symptoms produced by isolates of *Phytomonas medicaginis* var. *phaseolicola*. Phytopath. 34: 471–480. 1944.

[5] Kendrick, J. B., Jr., and J. C. Walker. Predisposition of tomato to bacterial canker. Jour. Agr. Res. [U. S.] 77: 169–186. 1948.

At periodic intervals stock cultures were compared with new isolates obtained from diseased plants. In most instances the virulence of inoculum from oil-covered isolates was comparable to that of new cultures. Two isolates, 3 and 30, placed under mineral oil in 1945, were as virulent when tested in 1948 as inoculum obtained directly from a diseased potato plant, and only slightly less virulent in 1949 than a fresh pathogenic isolate from tomato. The pathogenicity of the isolates has remained relatively stable. Although the disease indices for isolate 10 based on 14-day readings were lower in 1947 and 1948 than in 1946 and 1949, all the inoculated plants were usually dead 28 days after inoculation.

In other tests over a 2-year period with 11 different isolates representing a wide range in pathogenicity, even those isolates with low initial virulence did not become avirulent when stored under oil.

TABLE 1.—*Relative pathogenicity of isolates of Pseudomonas solanacearum tested annually for 4 years*

Isolate	Host	Disease index[a]			
		Sept. 1946	Sept. 1947	Sept. 1948	Oct. 1949
1	Potato	72	72	65	70
3	Potato	96	88	100[b]	88[b]
10	Tobacco	88	50	65	93
30	Peanut	88	84	83	90

[a] 14 days after inoculation.

[b] A subculture of isolate 3 made in September 1946 and placed under mineral oil was the source of inoculum for the tests in 1948 and 1949.

Although the factors influencing the longevity of bacterial cultures stored under oil were not studied, it was evident that complete coverage with oil prevented dehydration of the agar. When the agar was not completely covered, a gradual loss of water occurred, the oil level dropped as the volume of the agar medium decreased, and the desiccated cultures soon became nonviable. Although it was noted that growth of the bacterium was extremely slow under mineral oil, after several months the slant surface was covered with a thin film of bacteria.

In conjunction with these tests it has been possible during the past 3 years to use bacteria from oil-covered cultures for extensive greenhouse inoculations of tomato selections with the uniform elimination of susceptible lines.—North Carolina State College, Raleigh, North Carolina.

A Simple Method for Detecting the Presence of Phytophthora cinnamomi Rands in Soil.[1] E. J. ANDERSON. The need for a simple method of determining whether soils of pineapple fields are infested with the root- and heart-rotting fungus, *Phytophthora cinnamomi* Rands, has resulted in modification and combination of baiting and observation procedures which have proved useful. Several workers in Hawaii, notably F. P. Mehrlich, K. F. Baker, M. B. Linford, and the writer, contributed to the development of the

[1]Published with the approval of the Director as Technical Paper No. 195 of the Pineapple Research Institute of Hawaii.

present method. It has been used successfully for approximately 10 years in Hawaii and, in recent private communications, has been reported successful by W. A. Campbell in Georgia. The method is described here in the hope that others may find it, or modifications of it, helpful.

Small samples of soil are taken to include that between depths of about 1 and 6 in. The samples are protected from drying out by the use of waxed cartons, vapor-proof cellophane bags such as are used in quick freezing of foods, or tightly covered tin cans. Exposure to temperatures above 85° F. is avoided, since prolonged exposure to higher temperatures may prove lethal to the fungus. The best time to take samples is in the cooler and wetter season.

Baiting is done either by the use of pineapple crowns which have been allowed to root in clean water until roots are 1 or 2 in. long, or by means of heart leaves pulled from pineapple crowns or slips. In the latter case, only leaves having 1 in. or more of white tissue at the base are used, since the green portion is not susceptible to rapid infection. While other susceptible plants probably will serve, the writer has used only pineapple.

A pint- or quart-size Mason jar or similar container is filled with tap water, and 1 or 2 tablespoons of the well-mixed soil sample is added and allowed to settle to the bottom without stirring. The rooted pineapple crown is then placed in the top of the jar so that the roots are in the water. It is not advisable to have the roots touch the soil in the bottom, since this only complicates matters by allowing organisms which do not produce zoospores to grow onto the roots. If leaves instead of rooted crowns are used, they may be allowed to rest on the bottom so that the white bases are in contact with the soil. The jars are kept in the shade and at temperatures below 80° F., although exposure of an hour or two to slightly higher temperatures has not proved damaging. Temperatures of 70° to 75° F. are satisfactory.

After about 4 days, water-soaked lesions begin to appear near the root tips and the soft portion of the root soon becomes involved. Similar water-soaked spots appear at the base of bait leaves. Since water-soaked spots may be produced by other organisms, it is necessary to wait until fungus growth is visible on the affected parts for identification of *P. cinnamomi*. The affected parts are then removed, placed in a Petri dish of water or on a slide, and examined under a dissecting microscope or with the low power of the compound microscope. Presence of *P. cinnamomi* is indicated by typical nonpapillate sporangia borne singly on long sporangiophores, accompanied by proliferated sporangia with one to several successive sporangial walls telescoped within the original or outer wall. Confirmation of the presence of the fungus is obtained by isolating from bits of the infected tissue from the same baits by the usual plate procedure. In the writer's experience, it has been necessary to culture the fungus only infrequently, since in Hawaii no other fungus has been found to produce similar sporangia. The method has been used also for detection of *P. parasitica* Dastur and *P.*

palmivora Butler, but these can be confused easily. *P. cinnamomi* can be readily detected in mixture with the other species.

This procedure has been modified for use also in the estimation of effectiveness of chemicals for suppression of zoospore production and infection by placing desired dosages of the chemicals in the water after soil known to be infested with the fungus, or bits of agar cultures plus a small amount of raw soil, have been added. Obviously, it is useful for the production of sporangia for study. These can be produced in quantity without the use of the plant, however, by the procedure described by Mehrlich.[2]—Pineapple Research Institute of Hawaii, Honolulu, T. H.

Biologic Races of Ditylenchus dipsaci on Alfalfa.[1] OLIVER F. SMITH. The bulb or stem nematode, *Ditylenchus dipsaci* (Kühn) Filipjev, has a host range of over 300 plant species. Within the species there exist biologic races that infect only one host and others that infect several hosts. Alfalfa, *Medicago sativa* L., is a well known host plant of *D. dipsaci.* During the past 3 years the possibility of the existence of biologic races on this plant species has been investigated in conjunction with the alfalfa breeding program. The results of experiments, herein reported, show that at least two biologic races of *D. dipsaci* exist on alfalfa.

Infected buds of alfalfa plants have been collected from California, Nevada, Utah, and Virginia[2] and used as inoculum on plants resistant to nematodes collected in Nevada. Infected buds, which are greatly swollen and distorted, contain large numbers of the stem nematode in all stages of the life cycle and therefore make very satisfactory inoculum. Inoculations were made by spreading bits of infected buds among crowns of healthy plants in early spring and keeping them moist to favor movement of the nematodes and infection of alfalfa buds.

Inoculated plants were kept either in the greenhouse or in a cold frame for about 3 months after inoculation and then observed for nematode infection. Infected buds of susceptible plants had the characteristic swelling due to infection by the stem nematode. A plant was not considered a host to the nematode unless it could be determined by microscopic examination of infected tissue that the life cycle of the nematode was completed within the tissues of the plant.

Results from inoculation tests show that plant 28, a selection from the variety Nemastan, is susceptible to nematodes collected near Orland, Cali-

[2] Mehrlich, F. P. Nonsterile soil leachate stimulating to zoosporangia production by Phytophthora sp. Phytopath. 25: 432–435. 1935.

[1] Cooperative investigations between the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Nevada Agricultural Experiment Station.

[2] The writer wishes to express his appreciation to E. H. Stanford and L. G. Jones, University of California, Davis, for furnishing nematode collections from California; to Gerald Thorne, Division of Nematology, Salt Lake City, Utah, for sending collections from Utah; and to O. S. Aamodt, Division of Forage Crops and Diseases, Beltsville, Maryland, for sending a collection from Virginia.

fornia, but resistant to those collected near Patterson, Soledad, and Palmdale, California; Reno, Nevada; Salt Lake City, Utah; and Richmond, Virginia. Plants of Ranger alfalfa were susceptible to nematodes collected from all the above-mentioned locations.

Plant 28 is apparently only moderately susceptible to the biologic race of stem nematode which exists near Orland, California. Infected buds on clons of this plant have never shown excessive swelling, whereas infected buds of check plants of Ranger became greatly swollen and distorted.

Specimens of the nematodes collected at different locations were examined by Gerald Thorne[3], who reported that he could detect no morphological differences among the different collections.—Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Reno, Nevada.

Aster Yellows in Canna. FLOYD F. SMITH AND PHILIP BRIERLEY.[1] In August 1947 the writers examined canna varieties in a Delaware nursery for the presence of canna mosaic. The President variety was found free from mosaic[2], but a few plants of this and a number of other varieties had symptoms of more severe virus infection. Affected plants were much dwarfed and yellowed. Virescent flowers were noted on one plant. The aster-yellows virus was suspected as the causal agent. Symptoms of aster yellows were present in *Erigeron* sp. near the border of the canna field. The six-spotted leafhopper (*Macrosteles divisus* (Uhl.)) was not detected on canna by direct examination or by shaking the plants. No source material of the canna disease was collected, but symptoms duplicating those seen in the field have been produced in the President variety on transfer of this leafhopper from yellows-affected lettuce.

Aster yellows was transmitted from infected lettuce to the President variety of canna by *Macrosteles divisus* in two separate trials. The virus was subtransferred to China aster from one of the affected cannas in two trials (Table 1). In other experiments, not tabulated, *M. divisus* failed to transmit yellows from typically affected Cape periwinkle (*Vinca rosea*), either to canna or to China aster.

In cannas inoculated in August 1948, no symptoms of aster yellows appeared until growth was resumed in May 1949. At this time one of the plants exposed remained severely dwarfed and the young leaves had an irregular diffuse dull yellowing which turned to bronzing with age. Aster yellows was twice recovered in China aster from this plant by leafhopper transfer. The affected canna was dead 14 months after exposure to yellows.

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[2] Brierley, Philip, and Floyd F. Smith. Canna mosaic in the United States. Phytopath. 38: 230–234. 1948.

Cannas inoculated in June 1949, while small and in active growth, developed yellows symptoms on current-season growth in 2 to 4 months. Yellowing appeared first in the youngest leaves, which later became flaccid and withered. These effects progressed until the shoots died back to the soil line. Lateral shoots developed later (December 1949) were weak, yellowed, and dwarfed, and the weak second-season growth was dying 10 months after inoculation. The extreme dwarfing and yellowing in cannas in the field resembled these secondary effects in lateral shoots, suggesting that most of the symptoms in the field were second-season ones resulting from infections late in the previous growing season.

TABLE 1.—*Transmission of aster yellows from lettuce to the President variety of canna*

Source of virus	Plants inoculated	Date inoculated	Plants[a] affected	Date symptoms developed (1949)
Lettuce	Canna	Aug. 20, 1948	1/3	May 13
Canna	Aster	June 1, 1949	5/5	July 20
	Do	July 2, 1949	5/5	Aug. 1
Lettuce	Aster	June 23, 1949	5/5	July 20
	Canna	Do	1/3	Aug. 30
	Do	Do	3/3	Oct. 14

[a] Number affected/number inoculated.

This low incidence of yellows in cannas indicates that the disease is not a serious menace to the crop. However, the leafhopper vector feeds readily on canna, and some infections are to be expected in areas of high aster-yellows prevalence.—U. S. Department of Agriculture, Beltsville, Maryland.

An Aphid-Transmitted Virus Disease of Fuller's Teasel and Pincushion Flower.[1] WARREN N. STONER. In May, 1948, G. E. Altstatt observed a mosaic condition in a commercial planting of fuller's teasel, *Dipsacus fullonum* L., south of Sunnyvale, California, and M. W. Gardner collected specimens which were heavily infested with the rose aphid, *Macrosiphum rosae* (L.). In greenhouse experiments at Berkeley, lots of ten of these aphids were transferred to ten field-collected normal seedling teasel plants taken north of St. Helena, California, where the disease was not present. Infection occurred in one of the ten plants. Mechanical inoculations by the carborundum method of Rawlins and Tompkins[2] were made to ten of these field seedlings at the same time with expressed sap from the same specimens from which the rose aphids were taken. No infection occurred. Microscopic examination of portions of the affected field plants and experimentally inoculated plants failed to show any organism in the tissues.

[1] Investigations carried out in the Division of Entomology, University of California, Berkeley. The writer is indebted to Prof. E. O. Essig for identification of aphid species, and to Prof. J. H. Freitag for advice and assistance.

[2] Rawlins, T. E., and C. M. Tompkins. Studies on the effect of carborundum as an abrasive in plant virus inoculations. Phytopath. 26: 578–587. 1936.

The first symptom observed in teasel was a clearing of the veins on the basal portion of one side of the lamina in the younger almost fully developed leaves. Depending on the rate of growth and the age of the plant, this symptom developed in 4 to 8 days after insect inoculation. Two to 4 days later, asymmetry was observed in the affected leaves and veinclearing on one side (Fig. 1, B). Veinclearing and asymmetrical growth were not so marked in the next few whorls of leaves to develop, but a strong mosaic pattern plus progressive leaf malformation occurred (Fig. 1, C). Subsequent whorls of leaves were less mottled and more malformed (Fig. 1, D). In the last stages of the disease there was little left of the lamina (Fig. 1, E). Just before death of the plant, these filiform leaves were shortened, causing the plant to resemble in shape the flower of a spider chrysanthemum.

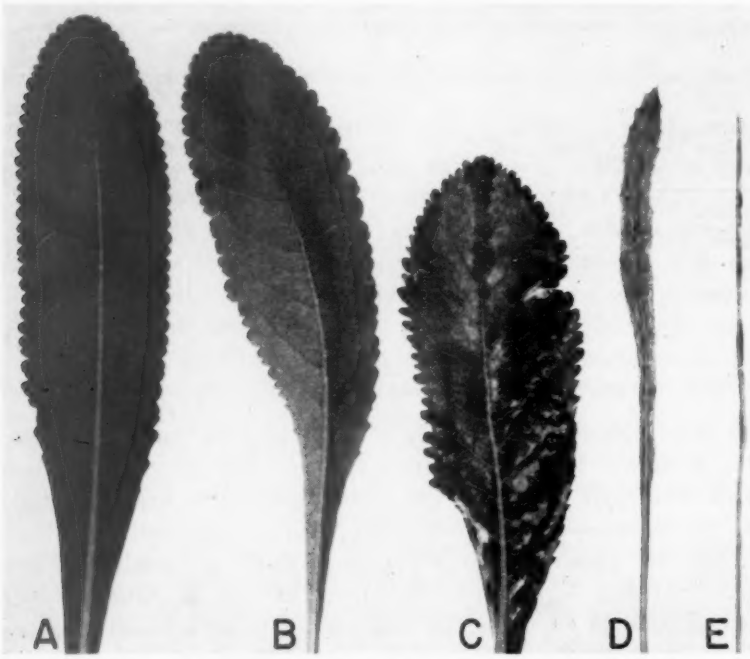

FIG. 1. Teasel mosaic. A, healthy leaf. B, asymmetry and veinclearing. C, mosaic mottling. D and E, filiform leaves.

Inoculations were made with aphids from periodically checked noninfective colonies, with the exception of the tests with rose aphids which were field collected from rose. All were with lots of five aphids each except as noted. The virus sources were teasel plants subinoculated from the original field plants. Equal numbers of checks were maintained at first, but later only half as many checks as inoculated plants were used. Twenty-five teasel seedlings were inoculated by each of the following aphid species: the rose aphid, *Macrosiphum rosae* (L.), the green peach aphid, *Myzus persicae* (Sulz.), the apple grain aphid, *Rhopalosiphum prunifoliae* (Fitch), and

the English grain aphid, *Macrosiphum granarium* (Kby.). Transmission occurred only in the series of plants inoculated with the green peach aphid and the rose aphid. Eight of the 25 plants in the rose aphid group, and 11 of 25 in the green peach aphid trial, became diseased.

Virus retention experiments were replicated five times with the rose aphid and the green peach aphid. The insects were fed for 24 hr. on diseased plants, then transferred, in lots of five, to ten healthy teasel seedlings. The insects remaining alive on each plant at the end of each 24-hr. period were transferred to another healthy seedling until all of the aphids were dead. The virus was transmitted to the first test plants in some of the series, but neither vector retained the virus long enough to transmit it in following serial transfers.

No literature was found indicating teasel to be a host of a virus. However, according to Smith,[3] a related genus, *Scabiosa,* was susceptible to cucumis virus 1. Carborundum and insect inoculations with *Myzus persicae* were made to attempt transmission of a stock culture of the cucumber-mosaic virus to fuller's teasel and pincushion flower, *Scabiosa atropurpurea* L. Transmission occurred by both methods in the pincushion flower series, but no symptoms could be seen in either series of the teasel plants.

Attempts were made to transmit the teasel-mosaic virus with the green peach aphid and by mechanical inoculation from diseased teasel plants to ten of each of the following plant species by each method: zucchini squash, *Cucurbita pepo* L.; sugar beet, *Beta vulgaris* L.; Turkish tobacco, *Nicotiana tabacum* L.; celery, *Apium graveolens* L.; pincushion flower, *Scabiosa atropurpurea* L.; and cucumber, *Cucumus sativus* L. Infection was obtained only in the pincushion flower series inoculated with aphids. Seven of the ten plants had symptoms of the disease. Symptoms in pincushion flower occur in the same sequence, but are not so pronounced as on fuller's teasel: asymmetry did not develop so strongly, the mosaic pattern was less distinct, and leaf distortion was less. Veinclearing, however, was more noticeable and persisted in all the affected leaves. The plants did not die so rapidly as fuller's teasel plants did. Thus only two species, *D. fullonum* and *S. atropurpurea,* have proved to be susceptible to the virus, both in the *Dipsaceae.*

It is believed the teasel-mosaic disease is caused by a hitherto undescribed plant virus. In general the characteristics of the virus conform to those of the genus *Marmor* of Holmes,[4] and the binomial, *Marmor dipsacum,* is proposed.

Marmor dipsacum n. sp.

This virus causes veinclearing followed by mottling and leaf deformation in *Dipsacus fullonum* L. and *Scabiosa atropurpurea* L. It is not sap-inoculable. It is transmitted by two species of aphids, *Myzus persicae* (Sulz.)

[3] Smith, Kenneth M. A textbook of plant virus diseases. 615 pp. London. 1937.
[4] Holmes, Francis O. Order Virales. The filterable viruses. Bergey's manual of determinative bacteriology. Ed. 6. Supplement No. 2: 1127–1286. Baltimore. 1948.

and *Macrosiphum rosae* (L.). It is not retained by the aphids. Descriptive habitat: California, U.S.A.—Everglades Experiment Station, Belle Glade, Florida.

Defoliation by a Rain-Favored, a Dew-Favored, and a Shade-Favored Disease. C. E. YARWOOD. The regular annual cycle of rainfall in California during the growing season provides some excellent examples of the relation of weather to plant diseases. In the San Francisco Bay area the average rainfall per month falls progressively from about 4 in. in January to 0.2 in. in June, 0.02 in July, 0.04 in August, and 0.5 in September, rising again to the seasonal maximum in January.[1] In many seasons June to September inclusive are free of rain.

FIG. 1. Left: Top portion of sycamore tree infected with sycamore anthracnose and showing bottom defoliation; photographed in Berkeley, California, June 28, 1942. Right: Prune tree heavily infected with rust and showing top defoliation; photographed in San Jose, California, October 16, 1942. In both cases the trees had the general conditions illustrated, but on a less aggravated scale, several weeks before the photographs were taken, and the photographs are intended to show a common seasonal condition, rather than a situation peculiar to the date indicated.

Imperfect fungi as a group,[2] and specifically anthracnose fungi,[3] are spread principally by rain, and only slightly or not at all by dry winds or dews. The rain functions to release the spores, disseminate the spores, and provide the free moisture necessary for spore germination. For this reason spread should be principally in a downward direction and the point of least infection should be the top of the plant. Sycamore anthracnose,

[1] Norquist, C. E. California Station. Climatological Data **50**: 145–155. 1946.

[2] Boughey, A. S. The ecology of fungi which cause economic plant diseases. Trans. Brit. Mycol. Soc. **32**: 179–189. 1949.

[3] Edgerton, C. W. The physiology and development of some anthracnoses. Bot. Gaz. **45**: 367–408. 1908.

which may be epidemic on wild and cultivated sycamores during rainy weather and is caused by the imperfect stages of *Gnomonia veneta*, is an excellent example. In California trees may be defoliated, except for the upper leaves, by this disease during the spring rainy season (Fig. 1), but infected trees later put out lower leaves which remain relatively free of the disease during the dry summer period. This escape of the upper leaves in an epidemic is also illustrated by Rankin,[4] but Rankin makes no reference to the phenomenon of bottom defoliation.

The uredial stages of rusts, on the other hand, may be more abundant in the absence of rain than in its presence,[2, 5] though free water is considered necessary for spore germination and infection. Urediospores are wind-disseminated and presumably well distributed over plant parts, but dews, because of their dependence on the outward radiation of the dew-collecting surface, occur principally on the upper and outer leaves. Logically, therefore, rusts might be expected to be more severe on the upper and outer leaves of large trees. That such is actually the case is indicated by observations of prune rust caused by *Tranzschelia pruni spinosae* on prunes and apricots in central California over several years. In epidemic seasons, which are apparently not correlated with late spring or early fall rains, the disease usually becomes conspicuous on prune in the late summer, and defoliates the tree from the top down (Fig. 1). This top defoliation is not due to naturally earlier maturity of the upper leaves, as it does not normally occur in the absence of rust infection. Furthermore, before defoliation begins, the lower inner leaves may be free of rust while the upper and outer leaves are heavily infected.

A third group of parasites, the powdery mildews, are commonly favored by shade and dry weather.[2, 6] Apricot powdery mildew, caused by *Podosphaera tridactyla*, illustrates the relation very well. It is rarely abundant early in the rainy season, but is commonly present on the lower and inner leaves of trees in the Santa Clara Valley of California in the dry late summer and fall, and sometimes many of the inner leaves of the trees drop. In vertical distribution, this type of defoliation is somewhat similar to that caused by sycamore anthracnose, except that in anthracnose, defoliation commonly extends to the lower outer leaves, whereas in powdery mildew the outer leaves even in the lower part of the tree seldom are infected. Furthermore, these diseases are distinguished by their season of occurrence, as already indicated.

These generalizations are believed to apply principally to tree diseases. —Division of Plant Pathology, University of California, Berkeley, California.

[4] Rankin, W. H. Manual of tree diseases. 398 pp. The Macmillan Co., New York. 1927.

[5] Yarwood, C. E. Relation of moisture to infection with some downy mildews and rusts. Phytopath. **29**: 933–945. 1939.

[6] Yarwood, C. E. Stimulatory and toxic effects of copper sprays on powdery mildews. Amer. Jour. Bot. **29**: 132–135. 1942.

Excess Callusing in Roses as Related to Flower Production and Vegetative Growth. E. S. McDONOUGH AND S. C. FOLL.[1] Since the early work of Melhus[2] and Riker and Keitt,[3] tumorous growths found near the graft union on roses, apples, and some other plants have been considered to fall into two main categories,—growths caused by bacteria, and overgrowths due to excess callus formation which has been attributed to a misfit of the cambiums of stock and scion. Some doubt seems to exist as to the productivity and vegetative growth of plants having nonbacterial callus knots, and, so far as the authors are aware, little statistical evidence has been published.

Many grafts of the rose variety Better Times on *Rosa chinensis* var. *manetti* stock, made in the winter of 1946 at the Holton and Hunkel Greenhouses, had excess calluses (Fig. 1). This seemed to afford an opportunity

FIG. 1. Callus overgrowth at the point of union of a graft of the rose variety, Better Times, on *Rosa chinensis* var. *manetti* stock.

to compare the productivity of roses having callus knots with roses having no excess callus. Adjacent benches of 684 plants of each type were planted near the center of a large greenhouse having nine benches on the same side of the central walk. Care was taken that the two lots were given, as nearly as possible, identical treatments as to fertilizing, watering, etc. Randomized planting of the two varieties was not practicable because of the time and expense involved.

No differences in vegetative growth have been observed. The plants were benched in June, 1946, and on February 22, 1947, the diameter of each branch at 4 in. from the soil was obtained for 118 plants of each type by a

[1] Respectively, Biology Department, Marquette University, Milwaukee 3, Wisconsin; and Holton and Hunkel Company, P. O. Box 1747, Milwaukee 1, Wisconsin.

[2] MELHUS, I. E. Crown gall of apple nursery stock. Jour. Econ. Ent. 19: 356–365. 1926.

[3] RIKER, A. J., and G. W. KEITT. Studies of crown gall and wound overgrowth on apple nursery stock. Phytopath. 16: 765–808. 1926.

systematic sampling of the plants in each of the four rows in the bench. The average diameter of the branches from stock not having excess callus was 6.287 mm.; that from heavily callused stock was 6.172 mm. The difference, 0.115 mm., was not considered to be significant since the standard error of the difference was calculated to be 0.124.

The 122 weekly cuts taken since October 1946 and including all roses cut up to the third week in June, 1949, were studied. The average number of roses cut per week from plants without excess callus was 395 (394.95901 actual); the average number cut from plants having excess callus was 410 (409.55737). The average difference was 15 roses. The standard error of the difference was calculated to be 50.86. The difference, 15, is exceeded by the standard error of the difference by more than three times and would, therefore, seem not to be significant.

It could be concluded that no difference in vegetative growth or flower production was observed; and that, judging from this experiment, roses with callus overgrowths are as desirable as normal callused grafts for bench roses.—Marquette University, and Holton and Hunkel Company, Milwaukee, Wisconsin.